CHOLERA:

INQUIRY

BY

DOCTORS KLEIN AND GIBBES,

AND

TRANSACTIONS

OF A

COMMITTEE CONVENED BY THE SECRETARY OF STATE FOR INDIA IN COUNCIL.

1885.

An Inquiry by E. Klein, M.D., F.R.S., and Heneage Gibbes, M.D., into the Etiology of Asiatic Cholera.

The nature and origin of cholera have, until recently, been subjects about which a great many speculations and theories have been put forward, in all of which the fundamental fact, viz., the demonstration and actual isolation of the cholera virus, was absolutely wanted.

The large number of observations made concerning the spread of cholera in India and the different visitations by cholera of Great Britain, the continent of Europe, and the Mediterranean, has yielded a number of facts, which have brought the different observers, as it were, under three well defined flags:—

1. According to some, cholera in its causation depends on no definite "entity," but is due to certain atmospheric and telluric conditions. Where these conditions prevail, as in Bengal, or where by some unknown change they obtain, as in the epidemics in Europe, cases of cholera occur. These cases stand in no causal relation to one another, but occur like ague or intermittent fever, independently of one another, but are dependent on a common cause of soil and atmospheric state. This theory, then, does not admit cholera into the rank of the infectious diseases, and it is hardly necessary to say that in Europe this theory has no great number of followers, since, with few exceptions, almost all medical authorities are opposed to it.
2. Other authorities maintain that, in the history of all the visitations by cholera of Europe and the Mediterranean countries, the character and course of every epidemic point clearly and unmistakably to the fact that cholera is dependent in a great measure on human intercourse, that each epidemic has had its origin in importation from a country where cholera cases had previously occurred, and that when once imported it is liable to, and generally does, spread to larger and larger areas; that if the first cases are isolated and sanitary precautions are taken the plague remains limited. This theory further says that cholera being communicable belongs to the group of infectious diseases, *i.e.*, diseases in which a virus having had access to the body of a person, therein multiplies to an enormous extent, and hereby causes the disease; that the morbid products, in the case of cholera, the vomits and evacuations, are charged with this new brood of virus, and that the smallest quantity of this is capable to start the malady in a new individual.

   The upholders of this theory, viz., that cholera is a communicable disease, group themselves again into two distinct classes,—(*a*) those that maintain that cholera is a contagious disease, *i.e.*, directly communicable from person to person, and (*b*) those that do not admit of direct contagion, but say that the substances voided by a patient in the vomits and evacuations are harmless while fresh, and do not as yet contain the actual virus, but acquire this power only after they have undergone certain changes, these changes being dependent, in a certain measure, on the medium into which the cholera dejecta pass, not every medium being suitable for producing this change; having undergone this change, this substance assumes the character of the cholera virus, *i.e.*, finding access to the human body, either through the air we breathe, or food, or water, produces cholera.
3. There is a third group of observers, headed by v. Pettenkofer, who, as is well known, is justly considered to be the greatest living authority on cholera. v. Pettenkofer is a localist with this distinction, that, judging from his earliest and latest writings, he does not admit of the evacuations of a cholera patient containing the virus at all, either potentially or actually, but considers the virus a product of an organism altogether extraneous to the body of a patient. This product is a kind of ferment created by that organism, only under certain favourable seasonal and local conditions.

The upholders of the first sub-section of Group 2 are spoken of as the contagionists, those of the second sub-section and of Group 3 as the localists.

There is no doubt that, as regards the observations on the spread of cholera and on the nature of epidemics, an immense amount of valuable work has accumulated in

o Y 9031. 817.—11/85. I 1857. Wt. P 1622. A

India and Europe; very capable and accurate observers have exerted themselves in this respect, and if the problem has not been solved as yet, it is doubtful whether any new observations in this particular aspect of the question will ever bring it to a solution. If it could be demonstrated with the same accuracy and to the same degree as has been done for some of the infectious diseases, notably splenic fever, tuberculosis, glanders, swine fever, erysipelas, viz., that the virus is a living entity, capable of multiplication within the infected body and thereby causing the disease, that the products of the disease are charged with the same living entities, that these organisms are capable of starting the malady when introduced into a new individual, then the whole problem might be said to be practically solved.

To have achieved this demonstration is claimed by Dr. Robert Koch, sent out in 1883 by the German Government to investigate the outbreak of cholera in Egypt and to study cholera in Calcutta. Koch has published in full his observations and conclusions in the "Berliner Klin Woch," 1884, No. 31. In the periodical reports, sent to his Government from Egypt and India, and still more in the above publication, the views of Koch on the nature and cause of cholera have been expressed with so much precision and definiteness, and Koch being justly considered a great authority on questions concerning the relation of micro-organisms to infectious diseases, it is not to be wondered at that a great section of the general and medical public take it for granted that the whole problem of the nature and cause of cholera is definitely and satisfactorily solved.

Now, what are the observations and conclusion of Koch? Koch failed to discover in the blood and other tissues of patients dead of cholera anything that could be identified as infective particles. He failed to find any kind of micro-organisms, and he therefore looked to the alimentary canal, which, as is well known, is the seat of grave disturbances. In the intestine he found this: in some cases the lower portion of the ileum, particularly just above the ileo-coecal valve, was of a dark brown colour, owing to hæmorrhage into the mucous membrane, which, in some instances, amounted to necrotic and diphtheritic changes, in these latter instances the contents being of the nature of a sanguineous dirty looking fluid. In other cases the redness was much slighter, limited to the margin of the solitary lymph follicles and Peyer's glands. In comparatively few instances, however, the mucous membrane showed very little change, being slightly swollen, less transparent than normally. The solitary follicles and Peyer's glands were prominent, the whole of the mucous membrane was slightly red, but there were nowhere capilliary hæmorrhages.

In these cases the contents of the intestine were, more or less, a fluid like rice water, in which were suspended the characteristic flakes, *i.e.*, masses of detached epithelium cells held together by mucus.

The last two sets of cases Koch considers as the typical and acute forms of cholera, and in such cases he discovered in the flakes of the intestinal fluid and in the reddened mucous membrane, particularly in those cases in which the margin of the Peyer's glands showed the characteristic redness, peculiar bacteria, which he called "comma-shaped bacilli." In sections through such a part of the mucous membrane, these "commabacilli" were seen to have penetrated from the free surface into the cavity of the Lieberkühn's crypts, and into spaces produced by the detachment of the epithelial lining of these crypts from their membrana propria. In other cases, other (putrefactive) bacteria could be seen penetrating into the mucous membrane, but the "commabacilli" were leading.

In acute typical cases of cholera the rice-water stools showed the commabacilli in greater numbers the fresher the stools. Koch found this condition to be characteristic of cholera; he saw it in Egypt, in Calcutta, and more recently in Toulon, and not finding the commabacilli in any other disease except cholera, he is led to attribute to them an important relation to cholera. Seeing that the more typical and fresher the case, the more pronounced the presence of the commabacilli is, seeing that in the most typical or acute cases the mucous membrane of the intestine is so crowded with the commabacilli that it represents, as it were, a pure cultivation of them, he concludes that these bacilli are the true cause of the disease.

To explain the manner in which these commabacilli produce the disease, he assumes that a chemical ferment is elaborated by the commabacilli, analogous to the septic or putrid ferment produced by putrefactive bacteria; this ferment, while at the same time killing and detaching the epithelium, is being absorbed into the general system, and then sets up all the symptoms constituting cholera. The more acute a case the greater the number of the commabacilli in the intestine, and consequently the greater the quantity of this chemical ferment produced. This—the presence of large numbers

of the commabacilli in the mucous membrane—is a point, the verification of which being obviously of essential importance, Koch has been able to ascertain to hold good in a large number of cases, for he found that the more acute the case the more conspicuously was the mucous membrane of the ileum a pure cultivation, and also the more affected the mucous membrane the more numerous were the commabacilli. Cases that do not run an acute course, *i.e.*, in which the disease passes the first stage, show on post-mortem examination less conspicuously the commabacilli, there being grave disorganisations of the membrane and consequently numerous putrefactive bacteria.

The commabacilli are then, according to Koch, the producers of the cholera poison, and the intensity of the disease is dependent in a direct ratio on the number of commabacilli present in the mucous membrane of the ileum. A very necessary conclusion from these statements is this, viz., that the rice-water stools, and particularly the mucus flakes suspended in the clear fluid (being charged with the commabacilli) are the vehicles of the virus, and the minutest quantity of these materials finding entrance into the small intestine of a person multiply in the mucous membrane, and produce the chemical ferment acting as the cholera poison; the more rapid the multiplication of the commabacilli the greater their numbers and the more acute and intense the disease; as long as their number is limited the amount of the ferment is too small to produce any other symptom except diarrhœa, but as their number increases the amount of the ferment increases, and a general infection of the system with all the typical symptoms ensues. A very remarkable fact stated by Koch is this:—If a mucus flake of the fresh rice-water stool, or of the fresh mucous membrane of an acute typical cholera case, be placed on a piece of linen or cloth, and this be kept damp, being placed in a moist cell or chamber (under a bell glass) at ordinary temperature, it is found that after 24 to 48 hours the commabacilli have increased enormously in numbers, later on putrefactive bacilli make their appearance, and by their multiplication gradually suppress the commabacilli.

Koch has cultivated the commabacilli artificially on sterilized media of different kinds; they grow well in milk, which is not curdled by them, in blood serum, particularly in nourishing gelatine, in broth, in Agar Agar with broth and peptone. Nourishing gelatine (composed of gelatine, peptone, and meat extract) is liquefied by the commabacilli; the Agar Agar mixture is not. The nourishing medium must not be too dilute. The behaviour of the commabacilli in nourishing gelatine differs from that of other bacilli, inasmuch as the growth forms peculiar funnel-shaped depressions. They grow well at ordinary temperature, but best at a temperature varying between 30 and 40 centigrades. The growth ceases below 16 centigrades. They do not form spores under any condition, but having reached in their multiplication a maximum, gradually shorten and die. The commabacilli require for their growth an alkaline medium. If the nourishing medium contains acid the bacilli cannot grow or multiply; and if free access of oxygen is prevented no growth of the bacilli can take place. The bacilli are killed by drying, and they are not killed by freezing to 10 centigrades.

Various substances inhibit the growth of the commabacilli. Iodine must be used in greater strength than in the case of other bacteria, similarly alcohol. Sulphate of iron in 2 per cent. mixtures, alum 1 in 100, camphor 1 in 300, carbolic acid 1 in 400, quinine 1 in 5,000, perchloride of mercury 1 in 100,000.

The commabacilli are about one half or two thirds the size of tubercle bacilli, are thicker, and they possess a characteristic curvature, which, as a rule, is not greater than that of a caraway seed, but occasionally amounts to a semicircle. After division the two bacilli may remain joined endways, their curvature being directed in opposite directions, and thus an S-shaped organism results. Occasionally, in gelatine, and particularly in broth, Koch saw them grow (by rapid division, and remaining joined endwise) into a sort of spirillum, not unlike a spirillum tenue.

These views of Koch, it is evident, favour in an eminent degree the theory of contagiousness, inasmuch as the commabacilli directly derived from the fresh evacuations are considered as the contagium vivum. Particles of these evacuations, linen and clothing soiled with them, provided these articles remain in a damp state, water, food, and other articles contaminated with the commabacilli can, under this view, directly convey the disease, if they find access to the alimentary canal, particularly to the small intestine. There exist a great many *a priori* difficulties in accepting this view of Koch. Foremost is the well known fact, observed over and over again, that direct contagion does not exist at all, or is of the greatest rarity; the attendants of a cholera patient, the persons living in the same room, physicians and nurses, persons occupied in handling and removing the evacuations of a cholera patient, are, according to almost all accounts, particularly

exempt; if the commabacilli were in reality the contagium, then it is impossible to understand why direct contagion should not be a very common thing. [It would be quite unjustifiable to maintain that the extraordinary panic which seized a section of the French and Italian nations on the visitation by the cholera in the summer of 1884 was caused by this theory of the commabacilli, but considering the authoritative position that Koch occupies, and considering the very decided way in which Koch, his Government, and the daily and most of the medical press gave expression to this view, it is not unreasonable to say, that that panic, although not caused, derived material support from it, for has it not been preached from day to day that the cholera evacuations are full of commabacilli, and that the commabacilli are the contagium of cholera? What, after this, is more natural than that the general public, reading such statements as coming from the highest authorities, should take up and spread the cry?] Again, it is well established by the researches of Pettenkofer and others, that between the introduction of the cholera virus into a new locality and the outbreak of the disease in the form of an epidemic there is always a considerable lapse of time; according to Pettenkofer the cholera matter introduced into a locality must, before becoming active virus, pass a certain stage of development in the soil, this soil must at the same time be of a definite character, and only after having undergone these changes and having had access to the system of persons can produce the disease. If the commabacilli of the evacuations were the actual *materies morbi*, such notorious immunity from cholera as is enjoyed by Versailles, by Lyons, Birmingham, by ships on the high seas, &c., could not easily be understood. In the several epidemics of cholera that visited Paris, thousands of persons fled from Paris into Versailles, some of them had contracted the disease in Paris, were ill in Versailles, but the disease did not spread in Versailles to other persons; if the commabacilli were the contagium, surely, when introduced into Versailles, there is no possible reason why, having taken a footing in Versailles, they should not have exerted the same power there as in Paris; the conditions under which in Paris the commabacilli can find entrance into healthy persons (linen and cloth contaminated with cholera evacuations, water, food, and other articles) are precisely the same as in Paris; the people in Versailles do not differ in respect of personal cleanliness, habits, &c., from those of Paris, and notwithstanding all this the disease did not spread in Versailles. The same can be said of Lyons and other places. A fact as well established as to have led to the adoption of radical and thoroughly efficient measures is this, that if into any ship lying off an infected country cholera cases are introduced, or cholera breaks out amongst the *personnel*, after removing the ship to the high seas cholera cases cease and the epidemic dies out; if the commabacilli present in the evacuations were the disease germs such a thing would be impossible, the whole *personnel* of such a ship, at any rate all susceptible persons, would become liable to attack. Another noteworthy observation, constantly acted on in India with conspicuous success, is this,—when in any military cantonment cholera cases occur, the troops are at once moved into camp and cholera ceases; surely no amount of shifting of the troops could in the least affect the commabacilli, for as long as there is one case of cholera amongst the troops, there would be available sufficient numbers of commabacilli to infect any number of persons.

Another great difficulty in accepting this theory of Koch is this: the commabacilli cannot, according to Koch, exist in acid media, and, therefore, when introduced into the stomach, this mode of infection being, according to Koch, the general one, they could not pass unscathed into the small intestine (the ileum being their true breeding ground). In an epidemic of cholera it is notorious that not only those become attacked by cholera that suffer from some gastric disturbance, for many persons previously in perfect health become subject to the disease, and a dyspeptic condition of the stomach ought to be the very best protection against cholera, seeing that in such a state it is hyperacidity which is present in the stomach, and therefore the commabacilli ought to have less chance. An empty stomach, that is, in the morning before any food has yet entered the stomach, would be the only condition in which the commabacilli could pass unscathed into the small intestine; but it can hardly be seriously assumed that this is the time when infection is carried out in all cases of cholera. If the commabacilli entered the stomach with the first morsel of food they would be attacked by the gastric juice, the secretion of which is immediately set going, and since they would have to sojourn in the stomach for some time like all the rest they would, no doubt, be killed. Thus we see that here are many weighty facts against accepting Koch's view as to the specific nature of the commabacilli.

In order to make good the proposition that the commabacilli are specific, it is necessary for Koch to show (1) that these commabacilli occur only and exclusively

in cholera; (2) that the commabacilli being absent from the blood and other tissues, must be present in the tissues of the small intestine in acute typical cases in enormous numbers, so as to cause the production of a correspondingly large amount of the chemical ferment—the direct poison; (3) that not only differ the commabacilli from other similar putrefactive bacilli as regards shape, but also as regards other characters, such as growth, behaviour towards reagents, &c.; and (4) that the commabacilli of pure cultivations are capable of producing the disease when introduced into the animal system. As regards No. 1, Koch maintains that the commabacilli occur only and exclusively in the intestine of patients suffering from cholera, and hence regards their presence as characteristic of cholera.

The examination by Koch of a number of cases of diarrhœa (infantile diarrhœa, diarrhœa due to other causes, dysentery, cholera nostras, and a variety of other conditions) invariably proved that commabacilli were absent.* [In a subsequent publication, Deutsche Woch, No. 45, 1884, Koch has modified this view, since he admits that comma-shaped bacilli occur also in other conditions.] And for these reasons Koch is of opinion that in doubtful cases the presence of commabacilli in the stools is of an infallible diagnostic value. From these statements of Koch we must differ most decidedly. In typical rice-water stools of cholera cases, however fresh, there occur, as is well known, a variety of micro-organisms:—(*a*) various species of micrococci, differing from one another in size of the elements in the mode of aggregation, some forming dumb-bells, and curved chains, and zoogloea, others forming only dumb-bells and sarcina-like groups, but not chains; (*b*) various species of bacillus subtilis, differing from one another in the length and thickness of the elements; (*c*) bacterium termo and lineola, this bacterium seems to possess in Bombay a wide distribution, since many artificial cultivations appear contaminated with it, but it is never missed in cholera stools; (*d*) vibrio rugula of Cohn (Beitr. z. Biol. d. Pfl. II.); but there are sometimes more than one species of them, differing from one another in the length and thickness of the elements; (*e*) spirillum, in all respects identical with the spirillum tenue of Cohn (spirillum denticola, spirillum Obermyeri), is often found in great numbers in cholera stools, particularly those that have been kept standing for several days; but we have seen also tolerably fresh stools containing a great number of them, although there were stools in which they were absent or only scarce; these spirilla, when abundant, present themselves in all shapes and lengths, from that of a single turn to that of three or four turns, either conspicuous by being twisted spirally or only more or less wavy; (*f*) *Koch's commabacilli;* these are caraway seed shaped curved organisms, with very slightly pointed or blunt ends; their length, measured like the tendon of the arch, varies between 0·0017mm. and 0·0026 mm.; they differ slightly in length and thickness in the same sample, but show greater differences in the amount of curvature. In the best examples the curve amounts to as much as half a circle, in most others it is only comparatively slight.

Now and then one comes across two commabacilli joined end to end, but so that their curves point in opposite directions, and nearly an S-shaped figure is produced.

Note I.—Since the name of commabacillus has been now universally adopted, we will retain it under protest. Its claim of being compared to a comma is in no way admissible, especially not to English readers, since an English comma is distinctly hook shaped; its resemblance to a comma as used by the Germans in writing is, however, more real. Nor is its claim to the name of "bacillus" more justifiable, since by "bacillus" a cylindrical or rod-shaped bacterium is understood. A great deal of misunderstanding has been caused by this unhappy comparison of Koch's cholera organism to a commabacillus; most English microscopists when first reading of it expected to find an organism of a distinctly hook-shaped appearance, and some went so far as actually to identify it with a hook-shaped something with thickened end. Thus, Dr. Bristowe states in the "Lancet" that some years ago, when engaged in a microscopic inquiry into cholera, he had actually seen (and drawn in his notes, unpublished till urged by Dr. Wilks) the "comma-shaped bacillus," and he figures a huge hook-shaped or rather club-shaped corpuscle with a thickened curved head, which he thinks corresponds to the cholera organism of Koch.

---

* Whatever Koch and his adherents may now state, there can be no doubt in the mind of any one who reads his pamphlet on cholera, that at the time he wrote it, *i.e.*, at a time when he had concluded his researches in Egypt, India, and France, he was not aware of there occurring comma-shaped bacilli in other conditions besides cholera. "Everywhere where I was able to come across a liquid containing bacteria I examined it in search "of commabacilli, but never found them in it" (see his pamphlet, p. 25); and "I therefore think I may say "positively that the commabacilli are constant concomitants of the cholera process, and that they are never "found elsewhere" (see his pamphlet, p. 25).

Dr. Bristowe's "organism" as much resembles the comma-shaped bacillus of Koch as a cylindrical epithelial cell of the intestinal mucous membrane resembles a bacillus anthracis; what Dr. Bristowe figures are, no doubt, distorted and injured epithelial cells.

Similarly, Dr. V. Carter, of Bombay, anxious not to be behindhand in claiming priority in having identified Koch's cholera organism, gave an actual demonstration of it to the Bombay Medico-Physical Society, and published also drawings and descriptions in the "Lancet," 5th September 1884. One of us had seen the commabacilli of Koch in specimens of rice-water stools, and artificial cultures prepared by Koch himself, and therefore knew exactly what they were like. On our arrival in Bombay, Dr. Carter was soon informed that in his specimens and drawings submitted for examination, the commabacilli of Koch were altogether absent, and that his drawings (the same as published subsequently in the "Lancet") showed a variety of spirilla tenua that have nothing whatever to do with the real commabacillus. The comma-shaped bacilli of Koch, as described sub. f., have no claim to the name of bacillus (Cohn), for under this name an organism is understood, which in its elements or single bacilli is straight, rod shaped. Owing to the shape of Koch's cholera organism, and owing to the fact that when, after the division of one, the two offsprings remain connected end to end so as to form an "S," it is more appropriate to consider it as a *vibrio* or spirillum, for a curved organism of this nature is considered to be a vibrio or spirillum, and is distinct from bacillus. From this and other facts presently to be mentioned, Koch's commabacillus ranks as a vibrio or spirillum. The commabacilli are then present in the rice-water stools of cholera patients, but their number is subject to very great variations; while in some they are easily found, in others it is difficult to meet with one. However, in the flakes of the fresh stools they are generally found in sufficient numbers to be easily detected, but we have seen typical fresh stools of acute cholera cases in which their number was so limited in the flakes that a careful search revealed only in one or the other field of the microscope a commabacillus. In stools of some hours and days they are not found so easily, being almost crowded out by the other organisms. In a few cases of cholera, which during the first 30 hours had passed a very large number of evacuations, some of them obtained during the second day of illness were almost clear watery fluid, in which were suspended minute mucus flakes; these flakes consisted almost entirely of hyaline mucus, and included only few cells; a very large number of commabacilli, but not to the exclusion of minute straight bacilli, were met with in the fluid and in the flakes. In other typical cases, during the first day of illness, the mucus flakes of the rice-water stools contained large numbers of other bacteria besides the commabacilli. (*g*) In some rice-water stools we have met organisms which are no doubt commabacilli, but differ from the ordinary typical examples of commabacilli in this, that they are distinctly semicircular, in some of them the ends are turned inwards, and then they represent rather more than half a circle. These semicircular organisms occur in two sizes,—(*a*) either such as correspond to long commabacilli with ends turned inward, or (*b*) such as correspond to very short commabacilli. This latter variety is sometimes met with in great numbers, and their uniform and minute size are very typical features in them (see Fig. 12). We were at first inclined to consider them as a distinct species, but a more careful analysis left no doubt that they are only a variety of the commabacilli. Both the large and small semicircular organisms grow, as is exemplified in the specimens, into corkscrew-shaped spirals, the former into spirals of much larger curve than the latter.

In cultivations in broth Koch found the cholera organism in forms which made him doubtful whether it is not rather a spirillum (see below), or at any rate an intermediate form between bacillus and spirillum; these forms (see his pamphlet, p. q., Fig. A a) are, however, quite reconcilable with the acceptance of the "commabacillus" being a minute form of vibrio. In cultivations in fluids (broth, liquefied gelatine, &c.) the commas grow through the S-shaped form into vibrios of several wave lengths, similar to a spirillum, but this is not therefore to be considered a true spirillum, since the typical organism, as shown in cholera material and in cultivations, is a single curved element or a couple of them arranged like an S.

Also in mucus flakes kept three days, of an acute typical case, the commabacilli were found growing into S-shaped and spirillar forms (see Fig. 13).

In this instance, although putrefaction was going on, there were mucous flakes containing enormous numbers of commas, S-shaped, and many-waved vibrios, all of the same thickness, but of slightly greater thickness than the commas in the fresh

flakes. They are easily distinguishable from the spirilla tenua one meets in putrid stools, for these latter are very much finer and stain differently in anilin dyes. So much for Koch's generalization that the commabacilli are inhibited in their growth and even destroyed by putrefaction.

This cholera bacillus, or, at any rate one that in morphological respects appears identical with it, occurs also in the stools of cases of diarrhœa.

In an epidemic of diarrhœa that occurred in the autumn of 1883 in Cornwall, the stools of patients contained besides various species of micrococci, of bacillus subtilis, and spirillum tenue, also curved organisms, which it is impossible to distinguish from the commabacillus of cholera stools; in size they are the same, in being curved they are the same, and, just as in the case with the choleraic commabacilli, some examples are either slightly pointed at the ends or blunt. They occurred not less numerously than they are sometimes found in cholera stools. In several cases of dysentery, besides the ordinary putrefactive organisms and spirilla tenua, commabacilli were found.

In cases of enteric catarrh commabacilli could be detected. In a case of chronic phthisis, of whom a post-mortem examination was made, the mucus of the small intestine, although free of any tubercle bacilli, contained besides other putrefactive organisms also commabacilli, and in this case they were so distinct that there was no difficulty in identifying them, and they were as numerous as in many cholera stools that we have examined. In the stool of a case of diarrhœa in a child, suffering from chronic peritonitis (February 1882), there are present in specimens stained with Spiller's purple numbers of commabacilli, which it is impossible to distinguish from choleraic commabacilli, in size, shape, and general aspect they appear identical. On the whole, then, we maintain, contrary to Koch's emphatic statement, *that commabacilli occur also in other cases of intestinal disease than cholera.*

And from this it must be clear that the statement of Koch, that the commabacillus is pathognomonic for cholera, and therefore is a valuable guide in deciding whether a doubtful case is or is not a case of cholera, is not borne out by our observations. A point on which Koch and his pupils lay great stress is this,—they admit now, since Koch's return to Berlin, that comma-shaped bacilli do occur in other conditions than cholera (Finkler and Prior found them in stools of cholera nostras, Lewis found them in the fluid of the mouth of healthy persons, see below), but they say the commabacillus of cholera stools is different in size from those occurring in other conditions, and what is much more important by artificial cultivation it can be shown that the choleraic commabacilli behave altogether differently from other commabacilli. As regards this second point, we shall return to this when speaking of the behaviour of the commabacilli in artificial cultivations, here we wish to state that the commabacilli occurring in our cases of intestinal catarrh above mentioned are no doubt slightly thicker than most of the choleraic commabacilli in rice-water stools, but—and this cannot be too strongly insisted on—a careful comparison of the choleraic commabacilli in different rice-water stools show undoubtedly differences in thickness, besides conspicuous differences in length. And more than that, cases were examined in Calcutta (typical and rapidly fatal cases) in which in one and the same specimen of the same rice-water stool the differences between the commas were most distinct, some being at least three or four times the thickness of others. Such differences in thickness, and still more in length, between commas are equally striking in the mucous flakes taken from the ileum of typical cases of cholera, as will be mentioned presently.

That the commabacilli should in some cases of cholera, particularly those with typical rice-water stools, with or without many mucus flakes, be very abundant may simply mean that here the commabacillus finds the most suitable conditions for growth, more suitable than any other bacillus, although, as a matter of fact, we have not found that, except in a few cases, it always predominates over other bacilli, particularly very short, thin, straight bacilli, to be mentioned below. The statement of Koch, that in acute typical cases the commabacilli are found chiefly and almost exclusively in the mucus flakes of the lower part of the ileum,—a statement borne out by our observations,—does not harmonize, it appears, with the assumption that the commabacilli are the cause of the disease, since, in several acute typical cases, there is no difference as regards the aspect of the intestine, the amount of fluid and flakes contained in the cavity of the intestine, and the anatomical changes of the membrane between the lower and upper portions of the ileum as well as jejunum.

Ad. 2.—In order to explain the causation of the disease by the commabacillus, Koch assumes that, it being absent from the blood and present only in the small intestine, a chemical ferment, which is the actual poison, is secreted by it, and on the

amount of this the severity and rapidity of the illness depend; in the typical acute cases a large amount of this chemical ferment is being produced, absorbed by the system, and therefore death rapidly ensues. And this, Koch states, is in accordance with the observation made by him, that in these instances the commabacilli are so numerously found in the mucous membrane itself, particularly in the lower part of the ileum, that this appears almost like a pure cultivation of the bacilli. If this were really the case, viz., if it could be shown that in acute typical cases of cholera not only the flakes composed of the detached epithelium and mucus, found in the cavity of the intestine and on the surface of the mucous membrane, but also, as Koch states, the superficial layers of the mucous membrane of the congested ileum are loaded with commabacilli and nothing else, this would be a remarkable fact, and there would be strong grounds for believing that the commabacilli must in some way or other be related to the morbid process, although it would not necessarily follow that these bacilli must, as a *conditio sine quâ non*, be the actual cause of the disorder.

Now, our observations are in direct opposition to these statements of Koch. It is difficult to explain how such a statement could have been made. Several cases of acute typical cholera were subjects of post-mortem examination. Death had followed in some within from 16 to 28, in others from 8 to 12 hours; the post-mortem was made in some within one, in others within half or a quarter of an hour. The ileum and, as a matter of fact, the whole of the small intestine was either slightly and uniformly injected and its mucous membrane slightly tumefied, the cavity both of the jejunum and ileum being filled with clear watery fluid in which were suspended large numbers of the typical flakes; there was no difference noticeable in this respect between the lower part of the ileum and the rest of the small intestine. In a few cases in the lower portion of the ileum the solitary follicles and Peyer's glands were distinct, and presented either a slight redness or only redness at the margin. Koch's statement that in acute typical cases of cholera the Peyer's glands and solitary glands of the ileum are enlarged, and on naked eye inspection already visible by a slight injection of their marginal portion, is not confirmed by our observations, since several acute typical cases came under our observation in which such a condition was not noticeable, that is to say, cases coming under the category of the pure typical cases of Koch, in which the mucous membrane ought to be almost "a pure culture of commabacilli."

The microscopic examination (carried out precisely after the same methods as used by Koch, *i.e.*, spreading out of a layer of mucus, drying it, and then staining with gentian violet, Spiller's purple or methyl blue, revealed in some cases in the flakes taken from the superficial layer of the membrane a goodly number of small bacilli, and also a considerable number of commabacilli, besides of course amorphous mucus and lymph corpuscles, and the detached cylindrical epithelial cells, some perfect, others degenerating and breaking down, as well as cells of the basement membrane; in other cases there were almost no putrefactive organisms present, only in one or the other field of the microscope could a fine short straight bacillus be detected, and in addition a few commabacilli, and in still other cases, besides the mucus, the perfect or degenerating epithelial cells, we could detect also, but at great intervals, one, two, or three commabacilli, and a similar or greater number of fine short straight bacilli. But in two cases very favourable for examination, death occurring after 12 and 18 hours respectively since the first symptoms, and post-mortem examination being made within half an hour to an hour, there were found in the flakes lifted off with a scalpel from the superficial layer of the mucous membrane remarkably few commabacilli; many fields of the microscope contained none, others one or two, but there were present, though in small numbers, the fine short straight bacilli; it must be stated that this does not refer to a very minute particle of a flake spread out on the cover glass in a thin film, but this and the previous statements refer to preparations in which a considerable mass of the flakes had been spread on the cover glass.

A point which appears to us of primary importance is this, that in some typical rapidly fatal cases when the examination was made soon after death the commabacilli were very scarce in the mucus flakes, while when the post-mortem examination was delayed, or when the patient remained *in articulo mortis* for many hours, the number of the commabacilli was much greater in the mucus flakes. Thus we find, for instance, as stated in the table on a following page, that the commabacilli were very scarce in Cases 14 and 16, and 23, post-mortem made after half, three-quarters, and $1\frac{1}{2}$ hours respectively, very abundant in Case 2, post-mortem after four hours, and when they were abundant in the lower part of the ileum in early post-mortem, also other bacteria were very abundant.

In the following is given a tabular statement of the occurrence of bacteria in the mucus flakes taken from the lower part of the ileum of typical rapidly fatal cases, the ileum was slightly reddened and filled with clear fluid in which were numerous typical flakes. The numbers attached to the cases indicate the number in the total series of cholera cases examined in Bombay and Calcutta.

1. Case 2.—Death after 40 hours. Post mortem made after four hours. Commabacilli abundant, small and large straight bacilli.
2. Case 11.—Death after 18 hours. P. m. after half hour. Commabacilli tolerably numerous; they vary in length, and particularly in *thickness*. Large straight bacilli exceedingly numerous; minute straight bacilli.
3. Case 14.—Death after 12 hours. P. m. after half an hour. Commabacilli very scarce. Few other bacteria.
4. Case 16.—Death after 18 hours. P. m. after three quarters of an hour. Very few commabacilli. Exceedingly numerous, small straight bacilli, singly and in clumps. Other kinds of bacteria.
5. Case 23.—Death after 20 hours. P. m. after $1\frac{1}{2}$ hour. Various species of bacteria; micrococcus, bacterium termo. Very few commabacilli; they are distinctly thinner than those of other cases. Minute straight bacilli in clumps.
6. Case 32.—Death after 27 hours. P. m. after two hours. All kinds of straight bacilli in great numbers. The small straight bacilli numerous. Commabacilli tolerably numerous; they are of different lengths and *thickness*.
7. Case 35.—Death after 13 hours. P. m. after quarter of an hour. Commabacilli tolerably numerous; large straight bacilli tolerably numerous. The small straight bacilli exceedingly numerous.
8. Case 48.—Death after 14 hours. P. m. after half an hour; great abundance of commabacilli, and also numerous minute straight bacilli.
9. Case 51.—Death after $9\frac{1}{2}$ hours. P. m. after one hour; various kinds of bacilli. The minute straight bacilli in extraordinary numbers. Commabacilli of three different kinds, distinct by their various thicknesses, some exceedingly minute, others five and six times as big, and a third variety corresponding in length and thickness to the typical commabacilli of other cases. The first variety in very large numbers, forming continuous masses. Numerous small semicircular commas, corresponding in size to the small variety of the above commas.

All these organisms were as numerous in the free flakes, as well as in those still on the mucous membrane.

Other cases, although typical and rapidly fatal, but in which the ileum did not contain the clear watery fluid with mucous flakes, are not included here.

This Case 51 is in many respects a very remarkable one, (*a*) on account of the uniform appearance of the whole ileum; (*b*) on account of the large number of typical mucus flakes suspended in the clear fluid which filled the cavity of the ileum; (*c*) on account of the commabacilli being present in, at least, three distinct varieties; it is impossible to assume that the commabacilli figured in fig. 12 at *a* should be the same as those at *b*, or as those at *d*; (*d*) on account of the great number of semicircular and also circular minute commas; (*e*) on account of the total absence of any bacteria from the mucous membrane itself, the epithelium of the surface being detached *en masse*. (*f*) On account of the enormous number of small commas and small straight bacilli, as also other kinds of bacteria already one hour after death, the illness having lasted only $9\frac{1}{2}$ hours.

Fine sections made of the mucous membrane of the above typical acute cases of cholera, after hardening the intestines in alcohol or Müller's fluid, particularly the first (also used by Koch), and stained in various aniline dyes (gentian violet, in several modifications, Spiller's purple, methyl blue, magenta, after Ehrlich's, Weigert's, Koch's, and other methods), revealed *the total absence* of commabacilli from the mucous membrane itself, from the tissue of the villi, from the Lieberkühn's follicles, and from the lymphatic tissue of the Peyer's and solitary glands; the epithelium of the surface of the villi having become detached during life has not generally kept its place in the hardened intestine, but in many places the epithelium of the surface, as well as that of the Lieberkühn's follicles, although loosened and slightly raised from the mucous membrane, had nevertheless kept its position and was fixed during the hardening; and in these places *the commabacilli or any other organisms are conspicuous by their absence*, they are nowhere to be found, they are simply absent.

A case very remarkable in this respect is the following. A woman servant died in the Medical College, Calcutta, of typical cholera, the illness having lasted only nine

hours and a half; she was taken ill at 10 a.m. on 19th November, and she was dead at 7.30 p.m. the same day. Post mortem was made an hour later. Body was extremely well nourished, the whole of the small intestine was slightly injected and filled with clear watery fluid, in which were suspended a large number of the typical mucus flakes. In those of the lower part of the ileum on microscopic examination were found very large numbers of exceedingly minute commabacilli, smaller than the typical ones of other cases, but there were present also many commabacilli, somewhat larger and of the size of the typical ones, and further a great many commabacilli conspicuous by their thickness and length, as much as six times as big as the first variety; there were present in large and small masses everywhere the small straight bacilli; finally, there were numerous micrococci in chains and dumb-bells, and thick straight bacilli singly and in chains. In fact, the mucus flakes seemed to be one mass of bacteria. Sections through the hardened mucous membrane showed that the epithelium of the surface was gone as a whole, but nowhere in the tissue (including the Peyer's glands) could there be detected a trace of commabacilli. If what Koch describes on page 6 of his pamphlet,—viz., the presence of large numbers of commabacilli between the epithelium of the Lieberkühn's follicles and the membrana propria, and extending into the cavity of the follicles, and into the surrounding lymphatic tissue of a Peyer's gland,—were a typical representation of what is the case in an acute and typical case of cholera, then we might reasonably expect to occur again in typical cases. But this is absolutely not the case. Our preparations were made after the same method as Koch's, they were well prepared and well stained, and if commabacilli had been present we should not have failed to bring them out, for in preparations of some cases other micro-organisms, such as micrococcus, bacillus subtilis present on the surface and in the superficial epithelium layer, are brought out very conspicuously. In two cases only were there present in sections through the Peyer's glands near the ileo-coecal valve commabacilli in some places around Lieberkühn's crypts, and also scattered here and there amongst the superficial parts of the lymph follicles. But besides the commabacilli, and in great majority, were straight bacilli, which with the commabacilli could be traced from the broken surface into the depth of the mucosa. As one of these is a good case in which commabacilli were found in the mucosa, but with a majority of straight bacilli; we will give for better estimation the history of this case. The patient, æt. 30, was attacked with vomiting and purging at 4.30 p.m. on the 6th October, he was admitted into the J. J. Hospital, Bombay, at 7.30 p.m. on 7th October. When admitted he was deeply collapsed, pulse imperceptible, features sunken, extremities cold, no urine. He died at 6 a.m. on 8th October. Post mortem at 8.30 a.m. The patient was evidently moribund from 7.30 p.m. of 7th October till 6 a.m. of 8th October, *i.e.*, for nearly 12 hours; in addition to this the post mortem was made two hours and a half after death; the temperature of the air was above 75 F. No wonder that under all these circumstances the tissue of the bowels should have become invaded by micro-organisms. In another case of acute typical cholera, where the post mortem had been made 14 minutes after death, but where the patient had been moribund from 9 a.m. till 3 p.m., sections through the hardened Peyer's glands and mucosa of the ileum showed the epithelium of the surface as well as that lining Lieberkühn's follicles bodily loosened and raised from the mucosa, but fixed in position during hardening. While there was total absence of commabacilli here or anywhere else in the mucous membrane and lymph follicles, there were nevertheless in some places on the surface minute groups of putrefactive bacillus subtilis, and from here they entered into the spaces resulting from the detachment of the epithelium of the Lieberkühn's follicles from the membrana propria. And even capillary blood vessels of the lymph follicles near the denuded surface, were found crowded with putrefactive bacilli and micrococci. In a third typical case, death after 10 hours, post mortem after half an hour, there were present numbers of straight putrefactive bacilli in the tissue of the villi and around the bottom of the Lieberkühn's follicles, but only here and there could a commabacillus be found close to the epithelium of the surface.

The preparation then, described by Koch on page 6 and figured on page 7 of his paper, is an exceptional one, and is not typical of cholera. As many typical cases as were examined by us, as often did we miss such a condition. It follows from this that the statement of Koch that in acute cases the mucous membrane of the ileum contains so great a number of commabacilli as to represent almost a pure cultivation of commabacilli is in direct opposition to the facts observed.

And if this statement of Koch is not in conformity with the facts, his inference, that the large number of these commabacilli produce a large quantity of the chemical

ferment and therefore an acute illness, is in no way justified, and his whole edifice as to the relation of the commabacillus to the disease having thus lost its chief support (viz., vast numbers of commabacilli supposed to be present in the mucous membrane in acute cases) falls to the ground.

As must be evident to every one who has carefully read Koch's description and arguments, the constant presence of crowds of the commabacilli in the mucous membrane in the lower part of the ileum in acute typical cases of cholera forms as it were the foundation for his whole theory of the relationship of these bacilli to the disease; and, therefore, if even in one single instance only of acute typical cholera (and with so much greater force in several such cases) it can be shown that the commabacilli are absent from the mucosa of the lower part of the ileum, then the theory that these bacilli are essential in producing the illness must be abandoned.

Some of the ardent supporters of Koch's theory, after it has been shown that the mucous membrane of the ileum or of any other part in the acute cases of cholera, provided the examination be made immediately or very soon after death, is absolutely free of commabacilli, might and probably will nevertheless cling to the commabacilli as the cause of cholera, saying: But the commabacilli are present in the cavity of the intestine, and although absent from the mucosa itself might nevertheless be the producers of the chemical ferment, seeing that they are present in such large numbers. As answer to this it may be repeated,—(1) that there are acute cases in which the commabacilli are very scarce indeed, even after the disease has well set in; that they should have been present in sufficiently large numbers in the lower part of the ileum before the symptoms appeared, in order to produce the large amount of chemical ferment which is to be absorbed—for this is what is meant by absorption of the chemical ferment, for no absorption can go on in an intestine during the attack itself, when the wall of the stomach and intestines discharge such enormous quantities of fluid as fast as they can—must be evident to every one to be an absurdity; an assumption of this kind would imply that the commabacilli are present in the fæcal matter in the lower part of the ileum before the setting in of the disease, and consequently they would have to remain here long enough to produce the virus, but for such an assumption there is not a tittle of evidence, and all our knowledge of the physiology of the intestine is against it; (2) that the whole of the small intestine presents in some actue typical cases the same appearances, viz., slight congestion, the cavity filled with clear fluid, in which are suspended the typical mucus flakes, and the great scarcity indeed of commabacilli in the flakes taken from the jejunum and upper part of the ileum; and (3) that the commabacilli are present only in dead tissues—for the mucus flakes are in all respects dead tissue, and they are found more numerously the lower down we go in the cavity of the ileum; these two facts point clearly to the commabacilli being putrefactive organisms. As mentioned on a previous page, the flakes typical of the rice-water like contents of the intestinal canal consist chiefly of perfect or degenerating epithelial cells and mucus, and, as has also been stated, there are present in them sometimes few, sometimes many, commabacilli, and also other bacilli.

Out of several typical acute cases there were two in which, with the flakes taken within half an hour after death from the superficial layer of the mucous membrane of the ileum, pure cultivations of commabacilli were established by one of us on bits of linen (after Koch) kept moist under a bell glass, as well as in test tubes containing sterilized nourishing material. Just as Koch has found it, after from 24 to 48 hours' exposure to the ordinary temperature (in Bombay this was about 80° F.), the mucous flakes placed on the linen were crowded with commabacilli, and the preparations obtained were identical with those figured by Koch in his memoir in Fig. 3, except that in our specimens the semicircular forms delineated by Koch were absent; after 48 hours various putrefactive bacteria, micrococci, and bacteria made their appearance and crowded the commabacilli out. In several other typical acute cases this mode of cultivation (*i.e.*, on damp linen) yielded no result, since from the outset numbers of putrefactive organisms were present. But also in one of the above two cases amongst a number of cultures thus established only one was successful for commabacilli, and from this it appears a little too sanguine on the part of Koch to recommend (see his Memoir, page 62) that in a case of doubtful nature, viz., whether cholera Asiatica or cholera nostras, all one has to do to decide it one way or another is, to make a microscopic specimen of a piece of linen soiled with the flakes of the evacuation or a culture on linen, and to see whether there are present commabacilli. This method, if successful, requires a good deal of luck; as a rule, in the majority of instances, commabacilli will be found absent, since in such

specimens, even when taken from typical cholera cases, the commas have not developed owing to crowds of bacteria of other kinds, for, as mentioned before, in the majority of instances the flakes of the rice-water stools contain (from the outset) already various kinds of other bacteria in great numbers.

As has been indicated above, the commabacilli seem to thrive best under conditions such as obtain in the lower part of the ileum, *i.e.*, mucus flakes suspended in a clear albuminous fluid, and for this reason it seems probable that these conditions being absent in the mode of cultivation on linen, the other common bacteria (micrococcus, bacterium, and various other common bacilli) find precedence.

The artificial cultivations in sterilized nourishing material have been achieved in several acute typical cases; in one the commabacilli that were present after 36 to 48 hours in large numbers on damp linen yielded the material for establishing cultures in test tubes; and also the flakes taken from the surface of the mucous membrane direct (within half an hour after death) introduced into test tubes yielded, out of a large number of tubes several that proved to be pure cultures of the commabacilli. In another case pure cultivations in test tubes were obtained in one out of a large number of tubes inoculated directly with a particle of a flake of the mucous membrane.

From these first cultures a large number of second, third, fourth, fifth, and so on successive cultures were established, and the character of the commabacilli when growing under various conditions could be accurately studied.

Now, it might be asked, does not this prove that the commabacilli in pure cases of cholera are the typical inhabitants of the cavity of the intestine, whereas the presence of other putrefactive organisms is an accessory? The answer to this is, No, not at all; what it proves is this, that it is possible to obtain pure cultivations of the commabacillus from the mucous membrane of the intestine; this necessitates the supposition that the particle used for inoculation was free of any but commabacilli. But a number of culture tubes were obtained from the selfsame intestine, in which there was a pure culture of a fine short straight bacillus; in these tubes evidently the particle used for inoculation contained the short straight bacillus respectively, but not the commabacillus. With patience, and the methods used for the isolation of the different species of organisms cohabiting in fluids or solids, there is not very great difficulty in isolating any species of bacteria, be they micrococci, bacteria, bacilli, or anything else (see Klein, "Micro-organisms and Disease"). And, as a matter of fact, pure cultivations have been in this way obtained of the bacterium termo, of the bacillus subtilis, and particularly of the fine short straight bacilli mentioned repeatedly as occurring in the rice-water stools. This was achieved after the method of Koch, viz., by diluting a particle of the mucus flake in a large quantity of nourishing material which at ordinary temperature is solid, but which can be liquified by warmth. In this case Agar Agar mixture (see below) was used, and after the particle of a mucus flake was diluted with one to two cubic centimetres of the sterilized and liquefied Agar Agar mixture, this latter is poured out in a thin layer in flat glass dishes and allowed to solidify. The dishes are covered up with glass plates and kept in moist cells. After from 24 to 48 hours or later, at a temperature of 75 to 80 F., various minute isolated specks are noticed in the glass dishes; each of these specks is a colony of generally one species of organisms, the different specks, if different organisms, show peculiarities by which they can be distinguished already with the unaided eye. In this way the commabacillus, the small straight bacillus, the bacterium termo, and other bacilli present in the fresh flakes, have been isolated.

Now, how do the commabacilli behave when artificially cultivated outside the body? do they exhibit characters by which they could be declared as different and specific from other bacteria, as is maintained by Koch (b. c.)? Besides the culture of commabacilli of the intestinal flakes on damp linen, the cultivations have been carried out in test tubes in sterilized nutritive material of the same composition and after the same methods as mentioned in Klein, "Micro-organisms and Disease," Chapter II.:—

Agar Agar and Brand's meat extract and peptone, alkaline.
Agar Agar and Brand's meat extract alkaline.
Agar Agar and peptone, alkaline.
Agar Agar and Brand's meat extract, neutral.

These materials were well sterilized and kept in sterilized test tubes, and plugged with sterilized cotton wool. The cultivations were carried on at ordinary temperature (about 80 F.), which proved quite sufficient for producing good crops already in 24 to 48 hours. All these materials are solid, and not liquefied by any of the bacteria that were cultivated.

The first and important result of the cultivations was this: commabacilli, micrococcus, bacterium termo and lineola, bacillus subtilis, obtained from the alimentary canal, behaved in exactly the same way in these materials; all yielded good crops in alkaline as well as in neutral media, when grown at ordinary temperature, except that all of them yielded more copious crops in the alkaline than in the neutral medium, and the commabacillus did not in this respect show any difference from the other bacteria. The best cultivations of commabacilli obtained were those made in neutral media, and, therefore, the statement of Koch, that the commabacillus shows this specific character that it requires an alkaline nourishing medium,—interpreted generally to mean that it does not live or grow well in any but alkaline media,—is not borne out by these observations. Koch lays great stress on the detrimental influence of acid on the life and growth of the commabacilli, and he uses this as an argument for his theory, that in healthy stomachs the commabacilli are killed, and cannot, therefore, pass in a living and active state into the small intestine.

Apart from the facts, (1) that the commabacilli in Koch's hands did actually grow and multiply on potato (of acid reaction), and (2) that a great many other organisms, such as bacterium termo, various micrococci and bacilli, both septic, zymogenic, and pathogenic, do not thrive in acid media, there is this important observation made in our cultures, that the commabacilli did actually grow and multiply in fluid nourishing media of a distinct acid reaction.

The commabacilli obtained from the flakes of choleraic ileum are not killed in media of weak acid reaction, for they live and, to a certain though limited extent, are also capable of multiplication in meat extract, peptone solution of distinctly though weak acid reaction.

As a second special character by which the growth of commabacillus is said to be distinguished from other bacteria is the peculiarity in its mode of growth in gelatine. The description given by Koch of the peculiar appearances presented by the growth of the choleraic commabacilli in nutritive gelatine is in all points correct. Koch states that in gelatine nourishing material the growth of the commabacillus shows this peculiarity, that when growing at a spot it gradually enlarges, and, unlike other bacilli, assumes an irregular granular or serrated outline. This character cannot be however, considered as in any way peculiar to the commabacillus only, *since some other bacilli* behave in a similar manner, *i.e.*, when growing on a spot or patch, show an irregular outline, and since groups of commabacilli do not always grow with irregular outline, but, as will be presently mentioned, with rounded outline. The commabacillus liquifies the gelatine, *so do other bacilli*, putrefactive and pathogenic ones, motile and non-motile ones, *e.g.*, bacillus anthracis, various kinds of bacillus subtilis, Jequirity bacillus, a short straight bacillus of the mouth of healthy persons, bacterium termo.

Koch states that in gelatine nourishing material contained in test tubes the commabacillus sown out at one spot on the surface of the material (*i.e.*, Fig. 5) grows into the depth with a characteristic funnel-shaped drawing inwards, liquefying at the same time the gelatine; but in connection with this, it must be remembered that almost each species of bacteria show certain peculiarities of growth by which an expert can distinguish them already with the unaided eye. Besides this the funnel-shaped drawing in of the surface by the growth is often absent, and moreover is also present in some growths of other organisms.

On Agar Agar meat extract, peptone, the commabacilli, when sown on the surface with the point of a needle or capillary tube, grow into transparent masses, with rounded or knobbed outlines, and sometimes with radiating lines on the surface (see figures). If the bacilli are deposited at a point, this is seen to increase into a circular spot, gradually enlarging into a round patch; the centre is thicker than the periphery, and hence a kind of central opacity is produced, *i.e.*, less transparency in the centre than in the periphery.

A very interesting difference is noticeable between the commabacilli carried on in neutral from those in alkaline media. In the former the bacilli, after from four to six days' growth, appear decidedly shorter, half the length of those carried on in the latter, their shape and curvature remaining distinct in both cases; some appear flat on one side, convex on the other. When growing in fluid which does not possess sufficient concentration, the commabacilli, as was also seen by Koch, after a certain stage of multiplication is reached, undergo degeneration, becoming granular, and finally altogether die; they fade away, and are lost to sight, and do not take to stains. The living commabacilli in cultivations are motile, single, S-shaped, or even more complex,

three or four remaining joined end to end, and they always move, owing to their peculiar shape, in a spiral; this, of course, is more pronounced in the S-shaped and complex ones than in the single examples.

Old cultures, some six or seven weeks old, in neutral Agar Agar, show that the commabacilli have a tendency to elongate, coiling and shrivelling up at the same time, so that forms are met with which look like more or less distorted spirilla.

Koch states (*l. c.* p. 20) that the commabacilli never form spores. The cultivations from flakes of the intestinal contents on damp linen, the cultivations of commabacilli in test tubes on alkaline solid material (Agar Agar mixture), proved after 24 to 48 hours the existence of commabacilli which differ from the typical ones in this respect, that they are about twice the thickness, some nearly flat on one side, convex on the other, and that they contained in their interior a spherical or slightly oval bright transparent droplet, which does not take the stain. In the short organisms there is only one about the centre, in the long examples there may be two or even three. And this appearance corresponds in certain respects to what is known of spores of other bacilli; in the well known cases of spore formation (bacillus subtilis, anthracis, &c.), the bacilli that form spores are known to swell up and to become thicker, and to form in the interior bright oval or spherical glistening spores which do not take the stain. And for spores these clear droplets could be easily mistaken, but further observations proved that this is altogether a different process, *i.e.*, vacuolation of the bacilli.

Now the objection might be urged; but are the commabacilli described and cultivated by Koch identical with those described and cultivated in this investigation. It is not likely that such an objection will be raised by any one who has carefully read this and the description of Koch's, and compared the commabacilli as figured here and in Koch's pamphlet, but seeing that a great many persons, unacquainted or only imperfectly acquainted with investigations of this kind, have asked innumerable unanswerable questions, and raised all kinds of objections to any criticisms unfavourable to Koch's view, it might be as well to answer such an objection. Well, first and foremost, the sources from which Koch obtained his commabacilli were identical with the places from which we derived our commabacilli, viz., acute typical cases of cholera; secondly, the locality from which Koch derived his commabacilli was identical with that from which we derived our commabacilli, viz., the mucous flakes of the lower part of the ileum; thirdly, the morphological characters of Koch's commabacilli are precisely the same as those of the commabacilli described here; an inspection of Koch's illustrations and ours, and a comparison of Koch's preparations with ours, leaves no doubt on this point; and lastly, the behaviour of the commabacilli grown on linen, on gelatine, and in other media is the same.

The examination of sections of the mesenteric glands, the kidney, liver, spleen, central nervous system, and the blood revealed no commabacilli. One of the most remarkable facts observed is this, the mesenteric glands in connection with the lower part of the ileum of several acute typical cases were examined carefully on fine microscopic sections, well stained with methyl blue, or gentian violet, or both, or Spiller's purple, and not a single commabacillus, not a trace of one, could be detected in them. If, as Koch maintains, the commabacilli in these cases are present in the earliest stages of the disease, and even before, in great numbers in the mucous membrane of the ileum (the mucous membrane and the cavity of the ileum being as it were a pure cultivation), it becomes absolutely unintelligible that no commabacilli should be absorbed and find their way into the mesenteric glands (for he assumes the presence of the commabacilli and absorption of the chemical ferment introductory to the outbreak of the symptoms of the illness). It is against all that is known of pathogenic organisms; for one great character of them is to be able to exist in the living tissues, and the least one ought to expect to find is their immigration, absorption, and presence in the lymphatic tissues of the mesenteric glands. But, as a matter of fact, they are totally absent in these organs.

[In the spleen there are certain microscopic lesions to be observed, consisting in death of patches of pulp tissue, these patches are irregular in outline and confluent; the healthy portions of the pulp become as it were permeated by dead issue; probably this change is caused by stasis of the blood in the venous radicles of these parts. It shows itself in stained sections as unstained patches, the elements of the pulp being indistinct and not taking the stain.

Some of the small arteries show hyaline degeneration of the inner coat, in consequence of which they become altogether occluded. In the kidney there is always opac swelling and granular degeneration of the epithelium of the convoluted urinary

tubes; in the capillaries of the Malpighian tufts there is sometimes hæmorrhage, always albuminous transudation into the cavity of the capsules of the Malpighian corpuscle. In the liver opac swelling of the liver cells and deposit of pigment in them.

In some of the lymph spaces granular matter may be met with taking the dye, and on superficial examination may be mistaken for organisms, but their irregular shape and unequal sizes prove them to be different.

The mucous, and particularly the sub-mucous, tissue of the intestine contain as a rule vast numbers of plasma cells, marked very conspicuously by their granules being stained conspicuously by the aniline dye, and generally of a different tint from the other tissues; thus, in specimens stained with methyl blue and Spiller's purple, the granules in the plasma cells are stained pink; in specimens stained with gentian violet they are deep blue. The same plasma corpuscles occur also in small numbers in the connective tissue of the kidney, particularly in that part forming the transition of the cortex into the medulla.

In the small intestine, besides the total loosening and detachment of the epithelium of the surface and of the Lieberkühn crypts, there is always enormous congestion of the blood vessels, and in some villi stasis.]

In a memorandum on the "Comma-shaped Bacillus," alleged to be the cause of cholera, Dr. T. R. Lewis, Assistant Professor of Pathology, Army Medical School at Netley, states that he examined sections of the small intestine of patients dead of cholera, in which no commabacilli were present, and that also in the cholera evacuations the commabacilli are sometimes extremely rare. From this he concludes Koch's statements as to the importance of the commabacilli entirely upset. Dr. T. R. Lewis had not at that time before him Dr. Koch's detailed account, and for this reason his negative observation proves nothing against the theory of the commabacilli. As has been quoted on a former page, Koch distinctly states that while commabacilli are present in varying numbers in the evacuation of cholera patients, they can be found always in very great numbers in the mucous flakes of the lower part of the ileum, and that in acute typical cases of cholera the part of the mucosa of the ileum surrounding the lymph follicles is characteristically and generally infiltrated with them, so much so that the mucosa appears like a pure cultivation of commabacilli, and that the further away from this part of the ileum the less numerous they are to be met with. Now, large portions of the ileum can under this statement be examined without finding any commabacilli, or only a few of them; and, nevertheless, the mucous membrane of the lower part of the ileum might be full of them. Besides, as Koch states, only typical acute cases show the uncontaminated appearances. Such being the case, it is clear that Dr. T. R. Lewis's negative observation proves not much against Koch's theory, for Dr. Lewis does not state to have examined the lower part of the ileum in acute typical cases. He might have examined hundreds of sections of the upper part of the ileum or of the whole jejunum, without finding any commabacilli. It happens that Koch is wrong with the lower part of the ileum (as has been minutely described on a former page), but that Koch's view might be right has not been disproved by those observations of Dr. T. R. Lewis. It is different with Dr. Lewis's statement of their occurrence in the mouth of healthy persons.

There is no doubt many not comma-shaped bacilli may, in dried and stained specimens, appear of a shape not unlike Koch's commabacilli, *e.g.*, in every specimen of tubercle bacilli, and glanders bacilli, and many others, one meets with individuals which are more or less curved, but that these are Koch's commabacilli no experienced observer would dream of thinking. Koch justly insists on cultivating the commabacilli of cholera patients, for only by artificial cultivations, *i.e.*, when large numbers of them become available, is it possible to be quite certain that one has to deal with a real and permanent form. Such being the case, the statement of Dr. T. R. Lewis that there occur in the saliva on dried and stained specimens bacilli which look like Koch's commabacilli does not yet prove that they are Koch's commabacilli. What he ought to have done is to cultivate them, and hereby to ascertain whether this is a real and permanent form, and if he could have been able to show that they behave in cultivations exactly in the same manner as Koch's commabacilli, then he would have been justified in drawing conclusions; but unfortunately he omitted to do so, and therefore his observations of bacilli which are curved, although quite correct, nevertheless do not possess full critical value. The same applies to the observations of Drs. Finkler and Prior (note in No. 36 of the Deutsche M., Woch, 1884), who have convinced themselves that in cholera nostras the evacuations contain commabacilli morphologically identical with those found in Asiatic cholera. This of course does also

away with Koch's statement of the diagnostic value of the commabacilli for cholera. But of course it does not prove that the two kinds of commabacilli are also physiologically identical.

Drs. Finkler and Prior have made certain statements as regards the life history of their commabacilli, which are so much at variance with what is known of these and other organisms, that their conclusions were not received with great favour; but nevertheless the fact remains that they saw commabacilli in cholera nostras, and that they have cultivated them. As has been stated afterwards by Koch these commabacilli of Finkler and Prior differ in an unmistakable manner from the choleraic commas in gelatine cultures.

What has been stated just now applies to all other criticisms of Koch's commabacillus, notably those by the French Commission, as published in the "Archive d'Anatomie and Physiologie." From all these criticisms this only is the positive result, that a commabacillus is not of that diagnostic value as first thought by Koch; but whether it is connected or not with the disease of cholera, is not proved one way or the other by all these observers, since they have not disproved, in fact have paid no attention whether or not in acute typical cases of cholera the lower part of the ileum, particularly the part of the mucosa surrounding the Peyer's and simple lymph gland, is infiltrated with them, is as it were a pure cultivation of the commabacilli.

Having thus far shown that some of Koch's statements as to the relations of the commabacillus to cholera are not borne out by the facts, it is now incumbent to show whether there occur in connection with cholera any organisms which, either in their distribution or in their general characters, could be reasonably associated with the cause of the disease.

The blood of cholera patients has been carefully examined in the fresh state, on stained specimens, and by cultivations; the blood was obtained according to the usual approved method from patients in various stages of the disease, from 10 hours after seizure to 48 hours, and in not one single instance could the presence of any kind of bacterium or other organism be shown to exist in the blood. The preparations examined fresh, those examined after staining with aniline dyes, revealed nothing that could be identified either as extraneous matter or as in any way indicating a specific morphological change; all assertions to the contrary must be put down as based on imperfect method of examination or insufficient acquaintance with the appearances of blood in health and disease.

Messrs. Sicard, Taxies, and others, forming a commission appointed in France to investigate the mode of action of cholera, have put forward statements with regard to the behaviour and morphological changes of the blood discs in cholera, which appear wholly without foundation; in fact we should like to place before this commission preparations of blood made of cholera patients within 10 hours, within 24 hours, and within 36 hours after the first symptoms of the disease set in, and at the same time preparations made of blood of perfectly healthy persons, and we challenge them to recognize the source of the preparations. That the virus of cholera is present in the blood, as maintained by these gentlemen, is an inference which has many probabilities, (see a future page), but that its presence in the blood can be recognised by the examination of the morphological properties of the blood discs is a proposition the correctness of which we totally deny. Equally unreliable are their statements with regard to the production of cholera in rabbits by injecting into their vascular system the blood of cholera patients in the algid stage. Rabbits are for such experiments totally unsuited, since they so readily succumb to septic infection after injection into their veins of a variety of substances, occasionally only the operation of the vein is followed by septic infection. That the death of the rabbits was due to cholera, and not, as is more probable, to septic infection, remains to be proved.

What has been stated just now does not in any way oppose the statement of these French authors, that in cholera the hæmoglobin of the blood discs becomes abstracted from the latter and destroyed; as a matter of fact the mucosa of the stomach, duodenum, and of the ileum (but in this latter to a much smaller extent), and particularly in the medulla of the mesenteric lymph glands, and in the tubules of the medulla of the kidney, there are found conspicuous signs of a wholesale destruction of blood corpuscles. In the muscularis muscosæ, and to a lesser degree in the muscularis externa, of the stomach, and particularly of the duodenum, there occurs an extensive deposit in the muscular tissue of blood pigment in the shape of fine brown granules, spherical droplets, and plates, so much so that all the muscular cells are beautifully marked as brown granular spindles.

In the medulla of the mesenteric lymph glands the lymph tissues contain vast

numbers of brownish particles of an oval or spherical shape, and of various sizes, from that of a minute granule to that of the size of a blood disc and larger.

Nor did the examination of the tissue of the liver, kidney, mesenteric glands and spleen, the medulla oblongata, and muscle, reveal the presence of any kind of specific bacteria, except in a few instances, when in the portal vessels a few bacilli of clearly putrefactive origin could be detected.

Numerous cultivations were made with the juice of the mesenteric glands, but no trace of bacteria was obtained, except in those tubes in which clearly and unmistakably putrefactive micrococci or putrefactive thickish bacilli had found their entrance. Thus then as regards the blood and tissues, the conclusion is imperative that no kind of bacteria are present in patients suffering from cholera.

In the small intestine, and particularly about the ileo-coecal valve, one finds in acute cases, dissected immediately or very soon after death, freely floating glassy looking clumps of mucus, which slightly differ from the ordinary epithelial flakes detached from the surface of the mucous membrane, or floating in the clear fluid. They resemble clumps more than flakes, and are more transparent; when examined under the microscope they prove to consist chiefly of mucous or lymph corpuscles, and of a few epithelial cells imbedded in a hyalin mucous matter. But the same lymph corpuscles may occur also, only not so numerous, in the ordinary flakes. These lymph corpuscles are always numerously present in those peculiar clumps, provided the examination is made *very soon after death*. After an hour and a half or two hours one misses them, since they easily become macerated and disintegrated in the intestinal fluid. They can be found also amongst the flakes of the rice-water stools, provided they are quite fresh, but then they are often obtained only in a fragmentary state. But the sooner the post mortem is made the more numerously they are found in those glassy clumps. Lewis and Cunningham in their reports on cholera have noticed them, and they correctly state that in order to see them the material must be fresh, *i.e.*, examined very soon after death. One misses their mention in Koch's paper altogether, be it that his attention was chiefly or wholly directed to the commabacilli, or, what seems more probable, his dissections were not made sufficiently soon after death. That this is the more likely explanation appears from the fact that when stained with aniline dyes many of these corpuscles contain some interesting things, as will appear presently; and had those corpuscles been present in Koch's specimens he could not have failed to notice their contents. If he had noticed them the probabilities are that he would have abandoned the commabacilli, since the contents of these mucous corpuscles are the only definite things that exist as regards bacteria, and they have a much more intimate relation to a tissue than the commabacilli. Examining these mucous corpuscles in preparations dried (after the Weigert-Koch method in thin layers) and stained with gentian violet, or Spiller's purple, or methyl blue, they present themselves as spherical, oval, or irregular corpuscles of about the diameter of ordinary white blood corpuscles, or larger, if swollen up. Each contains two or three deeply tinted oval, spherical, or angular nuclei. Their protoplasm is more or less hyaline, and they vary in size, inasmuch as many of them show signs of being swollen up or even in the act of disintegration, as is indicated by their faint or broken outline respectively. The best preserved spherical corpuscles are completely filled with very *minute straight bacilli*. Those that are slightly swollen show the bacilli more isolated, but still in many places in groups, and in those that are much swollen up and at the point of disintegration the bacilli are seen very loosely and irregularly scattered through the protoplasm, or on the point of leaving the corpuscle altogether. The accompanying figures 4 and 5 illustrate all these points. In the neighbourhood one always meets with the same minute bacilli scattered about. The appearances presented by these mucous corpuscles filled with the bacilli, and of those that have swollen up, and in which the bacilli are loosely scattered though them, are extremely striking, since the bacilli are stained deeply, whereas the cell substance appears homogeneous. Of course it is necessary to obtain these mucous corpuscles in a well preserved state, and for this reason they can be seen best in acute cases, *where the dissection is made immediately after death*. In stools, owing to the rapidity with which these corpuscles disintegrate, they are generally missed. And this is very probably the reason why Koch missed them, and for this reason it is justifiable to assume that Koch did not make his dissections sufficiently soon after death, a fact which has an important bearing on understanding his statement as to the presence of commabacilli and other bacilli in the mucous membrane itself. These lymph corpuscles are always to be met with in the glassy clumps and under the conditions mentioned above; but not in all instances does one find that they contain the same abundance of the small bacilli, for in some cases these latter were

missed in most of the well preserved corpuscles, and found only in those that had slightly swollen up or were on the point of disintegration. But in all instances the same small bacilli are found scattered in amongst the detached epithelial and lymph cells. There has not been a single case examined in which they were not found in the mucous flakes, in cases in which the commabacilli were very scarce, the small bacilli were not scarcer. In most cases they were met in larger or smaller groups and as isolated examples.

[As one amongst several interesting cases as regards the occurrence both of commabacilli and the small straight bacilli is the following:—E., aged 25, has been purging and vomiting since 12 o'clock in the night of 15th November; was admitted into the Medical College Hospital, Calcutta, on 16th November, at 10 a.m., under symptoms typical of acute stage of cholera. Died at 1.45 p.m., *i.e.*, a little over 25 hours after attack. Post mortem at 2.20 p.m. Ileum contains clear watery fluid, with glassy mucous flakes, numerous epithelial flakes. In the mucous membrane a few minute hæmorrhogic spots, not bigger than the point of a pin; Peyer's glands not visible. In the mucous flakes were large numbers of lymph corpuscles, some perfect and small, others swollen up; many of them contain the small straight bacilli in great numbers; besides these there were numerous large masses entirely composed of the small bacilli, but also commabacilli were everywhere to be found, although the small bacilli were in the majority. Cultivations made on linen from these mucous flakes yielded after 24 hours large crops both of commabacilli and of the small straight bacilli.]

These bacilli are of extremely small size, about half to two thirds the thickness of the commabacilli, and about one third or one fourth their length. They are straight, and appear pointed at each end; generally they are single, but also occasionally they form a chain of two elements. In the well preserved mucous corpuscles they lie closely packed together, apparently all single; in the large swollen corpuscles there are some in couples; and amongst those one meets free around and between the lymph corpuscles and epithelial cells there are a good many in couples and in small groups. It is not at all of rare occurrence to meet with mucous flakes of rice-water stools in which the corpuscles were found almost completely disintegrated; there were nevertheless found many groups of the small bacilli, from six to twenty.

Two questions present themselves in connection with these lymph corpuscles; (1) where do they come from? and (2) where do they get the bacilli from? There can be no difficulty in answering the first. It is well known that in all these places, where the highly vascular lymphatic tissue reaches the free epithelium of a mucous membrane, *e.g.*, the tonsils of the palate and pharynx, the lymph follicles of the pyloric end of the stomach and the duodenal part of the intestine, the solitary and agminated lymph follicles of the ileum, and those of the Peyer's gland of the lower part of the ileum and ileocoecal valve, lymph corpuscles pass (migrate) easily through the surface epithelium, and are discharged on to the free surface. This is the case already in the normal condition to a certain extent, and to a greater extent in the pathological state.

The mucous corpuscles found in the fluid of the mouth are those that have passed out from the superficial lymphatic tissue of the tonsils. In the Peyer's glands of the ileum one constantly meets these same corpuscles on their way through the surface epithelium.

The second question is more difficult to answer. From the fact that the bacilli are found inside and outside the mucous corpuscles, it might be said that the mucous corpuscles being endowed with amœboid movement while and immediately after passing out of the mucosa, are probably capable of swallowing the bacilli just as lymph corpuscles are capable of swallowing other granular matter; but against this might be urged, that the mucous corpuscles, having passed out of the mucosa, probably do not long retain their amœboid power: proof the rapidity with which they swell up and disintegrate in the watery contents of the intestine. The fact that the better the corpuscles are preserved the more numerous the bacilli, might be an argument either way, and, besides, several cases have been examined with this view, and only in one were the bacilli found plentiful within the well preserved corpuscles; they were absent, or almost absent, in the well preserved corpuscles of other cases, but were present in small numbers in those that had already swollen up or commenced to disintegrate. There is one other point which must be mentioned in connection with this,—it is the fact that, although these bacilli are not endowed with locomotion, it is not impossible that they settle on these corpuscles, and penetrate by active growth into them, finding in their protoplasm a good soil.

A very careful examination of fine microscopic sections of different parts of the

intestine, well preserved and well stained in the different aniline dyes, was made in order to trace, if possible, these small bacilli, isolated or enclosed in cells, from the lymphatic tissues of the mucous membrane outwards, but all in vain. No trace of them could be found in the lymph corpuscles or any other part of the mucous membrane, neither in the stomach, intestine, mesenteric glands, blood, or any other tissue.

On the whole, then, although these bacilli looked very promising at first as regards their connection with the disease, they had nevertheless to be abandoned, and had to be regarded, like the commabacilli, as something extraneous, present only in tissues practically dead in the cavity of the alimentary canal. But if any one wishes to urge that these small bacilli are probably connected with the disease, there would exist for such a view at least as much, if not more, justification than for Koch's commabacillus, since these small bacilli are found in some elements derived from the tissue of the intestine; the commabacilli are not, and are always present in the mucous flakes and in the intestinal contents, at any rate in acute cases, and post mortem examination being made soon, as often and as numerously as the commabacilli. In the watery vomit, when copious, of acute cases, these small bacilli are generally not missed, chiefly as isolated individuals or in small groups.

And in the same way, if not more so, one might further urge that they are quite capable of forming some kind of chemical ferment, which, when absorbed, produces the disease. All this could be said with the same justification of these small bacilli as Koch has done of the commabacilli, and such a theory would rest on a basis not a bit weaker than the one which Koch's theory of the commabacillus rests on. Any one who feels satisfied with such evidence is quite welcome to use it.

These small bacilli have been cultivated by one of us in the same way as the commabacilli, on linen kept moist by filter paper under a bell glass, on mixtures of Agar Agar, meat extract, and peptone, alkaline and neutral, and its characters have thus been studied. They grow well at ordinary temperature (75° to 82° F.), so that already, after 24 to 48 hours, considerable masses become available; of course they grow much more rapidly at higher temperatures (90° to 102° F.), and they grow like the commabacilli and other bacilli much better and more copiously in alkaline than in neutral media.

The appearances presented after inoculation of the material in test tubes is very much like that presented by the commabacilli; from the point of inoculation the growth spreads in the form of a flattened or filmy rounded whitish mass, its outlines uneven or knobby. Growing on linen and on Agar Agar mixtures (solid), the bacilli are seen singly or very often in chains of two or dumb-bells, the single bacilli are of the same small size as those mentioned above, but many of them grow to somewhat greater length in the cultivation than in the fresh material. After 24 to 48 hours' growth some of them begin to show the formation of spore in the shape of a bright glistening spherical granule, the substance of the bacillus gradually becoming pale, not staining, and ultimately altogether fading away, so that only the spore is left. After several days' growth many of the bacilli, which have not formed spores, become pale, stain very faintly, and gradually fade altogether away. This change indicating the degeneration and death of the bacilli differs in no way from what was observed of the commabacilli, and described on a former page. Growing in gelatine they do not liquefy the material, and form clumps of a whitish appearance.

When in Egypt and Calcutta, Koch performed a large number of experiments by feeding, subcutaneous and intravenous injection as well as injection into the duodenum with rice-water stools, and with pure cultivations of commabacilli, on rodents, carnivorous animals, and monkeys, and obtained no result, and his inquiries amongst the people led him to the conclusion that no case was known of a domestic animal having taken cholera, and he therefore came to the conclusion that cholera is not transmissible to the lower animals. He made, however, the observation that animals (rodents) may die of septicaemia after inoculation with rice-water stools, and that the commabacilli are capable of multiplication within the animals inoculated, without, however, producing cholera. Since his return to Berlin he maintained that he has been able to confirm the assertions of Nicat and Rietsch, viz., that injection of the commabacilli into the duodenum of dogs and guinea-pigs led to death with multiplication of the commabacilli, and he therefore considers it proved that the commabacilli are pathogenic organisms.

A large number of experiments were performed by one of us on rodents, cats, dogs, and monkeys by feeding, by subcutaneous, intraperitoneal, and intravenous injection,

and by injection into the cavity of the upper part of the small intestine, of mucous flakes of the ileum of typical acute cholera, and of pure cultivations of choleraic commabacilli and the small straight bacilli; the results of these experiments are described in the following pages.

Experiments on Animals with Choleraic Evacuations, and Cultivations of its Bacteria.

Various experimenters have tried to communicate cholera to the lower animals, but without success. Thiersch, Sanderson, and others thought to have succeeded in white mice by feeding them on bits of paper steeped in choleraic evacuations, but it was pointed out by Ranke that similar results are obtainable by feeding them on paper that had not been so steeped. Koch has made a large number of experiments, both by feeding on and inoculation with choleraic evacuations and with pure cultivations of commabacilli of cats, dogs, monkeys, mice, guinea-pigs, rabbits, and rats, under the most varied conditions, but without positive results. When a disease is produced in guinea-pigs, rabbits, and mice, it is not cholera but septicaemia, and Koch made the important observation that in mice the animals inoculated with the commabacilli die sometimes of septicaemia, but not of cholera, and show then commabacilli in the blood.

Another important observation of Koch is the one according to which the commabacilli are capable of *multiplication when introduced directly into the small intestines, but without producing cholera.*

It seems clear from these observations of Koch that the commabacilli can propagate themselves and thrive well in the bowels, and even in the blood, but without producing cholera. In connection with this it is most important to notice that there have been made several similar observations with regard to other putrefactive bacteria (*vide* the bacilli of papayin solution, the bacilli in Jequirity infusion). In the experiments of Professor Rossbach (Centralbl. f. d., medizin, Wiss. 5, 1882), injection of a solution of papayotin into the veins of rabbits is followed by death of the animals. The blood teems with bacilli; these bacilli are derived from spores that had been present in the solution of papayotin. But death of the animals is produced in exactly the same way if those spores are, previous to the injection, removed by filtration (Dowdeswell, Practitioner, 1883). Consequently, those bacilli have nothing to do with the disease and death of the animals. Their development is due to the blood being transformed by the papayotin poison into a medium suitable for the development and growth of these bacilli.

In the same way, an unfiltered Jequirity solution contains the spores of bacillus subtilis, and, if poisoning is produced by injection of such a solution, the tissue and the blood may become a suitable soil for the growth and multiplication of these bacilli (Salomonsen). But the disease and death of the animals have nothing to do with these bacilli, since a perfectly sterilized Jequirity infusion produces disease and death in precisely the same manner as one that has not previously been deprived of these spores.

The experiments performed by Dr. Richards on a pig have been fully considered by Koch, and the conclusion becomes inevitable that the cause of death in the pig was due to a toxic principle present in the choleraic evacuations, similar to what is known to be the case in typhoid and other evacuations.

A very curious illustration of how easily investigators are led into error is furnished by the description given by Nicati and Rietsch (as quoted in the "Fortschritte der Med." Heft 19, Beilage, copied from "Semaine Medicale," p. 370) of experiments on dogs, in whom the chief bile duct had been ligatured before introducing into their intestines choleraic evacuations or pure cultures of Koch's commabacilli. These dogs were said to die after one or two days with choleraic symptoms. The intestinal contents were a creamy fluid (see below), with many detached epithelial cells. In these contents the commabacilli were found in great numbers.

If these observers had ligatured the chief bile duct in many dogs, they would have found in some cases death to occur in the same way, and the intestine filled with the same creamy contents. If, now, a loop of this intestine had been cut out, and commabacilli had been introduced into it, the commabacilli would have found herein a good soil wherein to multiply. And such experiments are seriously put forward as proving that cholera is transmissible to animals, and that the commabacilli are the *materies morbi*, because they had been found capable of multiplication in the cavity of the

intestine. It would have been very surprising if, under such conditions as the above, the commabacilli had not multiplied, because the intestine was made diseased, and the commabacilli grow well in the intestinal contents, mucus and detached epithelium.

Dr. C. Friedländer (*ibidem*), who seems to be very enthusiastic about these experiments, shows a singular want of judgment in thinking that, because in cases of fully developed cholera the intestinal contents and evacuations are free of bile, therefore a necessary condition for the success of the experiment in animals is the exclusion of bile, *i.e.*, the ligaturing of the chief bile duct as done by the French experimenters. With equal justice he might argue that, because in cholera there is suppression of urine, therefore a necessary condition for the success of the experiment is the ligaturing of the ureters. How many cases of cholera has Dr. Friedländer seen? Have not all, before the disease actually set in, *i.e.*, during incubation, shown coloured fæces? The absurdity of such an argument is self-evident. But the want of judgment in the case of the French experimenters, and perhaps still more so in their critic, Dr. C. Friedländer, becomes quite inexplicable if in the same article we read that the experiment with the commabacilli succeeded equally well in guinea-pigs, in whose stomach and intestine the commabacilli had been introduced without previous ligature of the bile ducts. The animals in which by opening the peritoneum the intestine has thus been operated upon died from "choleraic" symptoms.

Koch having then ascertained, (*a*) that cholera is by no means transmissible to the lower animals, (*b*) that if the animals (mice, rabbits, guinea-pigs) die it is from septicaemia, and not cholera, and (*c*) that the commabacilli are capable of multiplication within the intestine of animals without producing cholera (see page below), it must seem most astounding to all who have followed his statements to find that Koch suddenly (Deutsche Medic., Woch, No. 45, 1884) asserts that he has been able to convince himself that cholera is transmissible to animals.

Encouraged by the experiments of Nicati and Rietsch, he has injected into the small intestine of several dogs, but without ligaturing the bile duct, small quantities of pure cultivations of commabacilli, and found that, with few exceptions, the animals died between from $1\frac{1}{2}$ to three days, the small intestine was reddened, and its cavity contained a watery fluid; the commabacilli injected had greatly multiplied. Koch does not state under what symptoms the animals died. It is to be assumed that if they had shown the symptoms of cholera, Koch would not have omitted to state it, but not saying so they evidently did not show any of those symptoms which indicate cholera, *i.e.*, vomiting, purging *with rice-water stools*, cramps, *great fall of temperature*, and *suppression of urine*.

The two statements of Koch, viz., the first in his full report, and the second recently made, as to the production of cholera by commabacilli in animals, are in direct opposition, and it is quite impossible to reconcile them.

There seems, however, one way to explain this remarkable change of Koch's, and it seems this,—v. Pettenkofer challenged Koch to supply him with pure cultivations of the commabacilli, as he (Pettenkofer), with other medical men in Munich, are quite ready to swallow any quantity of them. Koch could not easily leave unanswered such a challenge, and it was, therefore, very urgent to show that such an experiment would be unnecessary, since the pathogenic properties of the commabacilli can be tested on animals.

Dr. Van Ermengen (Berliner Klin, Woch, Dec. 1884), while confirming Koch as to the distribution of the commabacilli and their mode of growth, was also successful in producing death in animals inoculated with the commabacilli. That these animals should have died from septic infection, or in consequence of the operation, does not seem to have occurred to any of these observers. This might easily have been thought of; the operation of getting at the duodenum, after the opening of the peritoneal cavity, is not a light one, and, in addition, these experiments were not done thoroughly antiseptically. To crown all this no control experiments whatever seem to have been performed. If they had done so, *i.e.*, if they had opened the peritoneal cavity of dogs, and still more of rodents, without antiseptic precautions, and if they had then pulled out the duodenum, an operation always involving a considerable amount of mechanical injury to the intestine, and if they had omitted altogether to inject the commabacilli, they would have obtained death of many of the animals owing to septic poisoning, or in consequence of the mechanical injury. The diarrhœa and intestinal disease, present after such an operation, obviously cannot be placed to the account of the commabacilli, since the same symptom is observed in a very large per-centage of cases of pure septic poisoning.

A number of experiments were performed on monkeys, cats, mice, rats, and rabbits. The results are these,—

1. Two monkeys were fed* with rice-water stool abounding in commabacilli on the same day that the stool was passed, and again after having been kept for 24 hours. No result.

2. Fed the same two monkeys with pure cultivation of commabacilli. No result.

3. Fed the same two monkeys with cultivation of commabacilli on linen. No result.

4. Fed four fresh monkeys with rice-water stool mixed with milk, after having been kept spread out on bread for 24 hours. No result.

5. Fed the same four monkeys with rice-water stool mixed with milk, after having been kept spread out on bread for 48 hours. No result.

6. Fed the same four monkeys with watery choleraic vomit, mixed with milk, after having been kept spread out on bread for 24 hours. No result.

7. Two cats were fed with fresh rice-water stool. No result.

8. The same two cats were fed with rice-water stool kept for 24 hours. No result.

9. Fed two fresh cats with rice-water stool mixed with milk and bread, after having been kept for 24 hours. No result. (One of the cats was found dying 16 days after. The animal had fallen off its food during the last four or five days, but otherwise had showed no symptoms. On post mortem the stomach and intestines were found perfectly normal, so was the liver, spleen, lungs, and blood. Both kidneys were enlarged, white, and fatty degenerated.)

10. With rice-water stool kept for 24 hours, and then mixed with milk and bread, fed four white mice, 1, 2, 3, 4. Fed them again with same stool having been kept for further 24 hours.

Mice Nos. 1 and 2 were found dead on the third day after the second feeding. No symptoms observable during life. On post mortem examination it was found that the small intestine was distended with mucus, lungs were found inflamed; no other change. The microscopic examination of the heart's blood revealed the presence of numerous longish straight bacilli. The contents of the small intestines showed, besides epithelial cells in various stages of disintegration, large numbers of longish straight bacilli (b. subtilis), many of them with spores, and numerous micrococci. No commabacilli of any kind. Mouse No. 3 was found dead on the fourth day. Lungs were much inflamed, small intestines distended with creamy fluid; this fluid contained numbers of epithelial cells and whole groups of epithelial tubes, evidently the lining of Lieberkühn's crypts, and besides straight bacilli (bac. subtilis) large numbers of beautiful comma-shaped bacilli of at least two (possibly three) varieties differing from one another very markedly in length and thickness. They were isolated or in couples, and then S-shaped; they were markedly pointed at their extremities.

The smaller variety were a little larger than the commabacilli of cholera stools, but were conspicuous and different by their very tapering and pointed ends. The blood of the heart contained various kinds of straight bacilli, and a fair number of commabacilli of the same different varieties as those in the intestinal mucus.

Such a condition, namely, lungs inflamed, intestines inflamed and distended by creamy mucus, is a condition familiar to every one who has seen many cases of septicaemia, both in mice and rabbits, occurring after experiment, and, in not by any means rare instances, spontaneously. To consider the death of the above mice as due to cholera, and their post mortem appearances as indicating cholera, would be absolutely unwarranted. It so happened that at the same time two mice died that had not been the subject of any experiment, having been kept altogether separately. One interesting fact resulted from these last experiments; it is this, that commabacilli of different varieties and different from the human commas were found in the intestinal mucus and in the blood of an animal that could not be said to have been affected with cholera.

Experiments made with Intestinal Mucus and with Blood of these three Mice, Nos. 1, 2, and 3.

(*a*) Mixed intestinal mucus (fresh) of mouse 1 with milk and bread, and fed with it two mice (11 and 12), and two rats (5 and 6).

---

* All "feeding" was done on empty stomach.

In the same way fed with intestinal mucus of mouse 3 two mice (17 and 18), and two rats (7 and 8).

The mouse 11 was found dead three days after; there was no distinct anatomical change in any organ.

The mouse 12 was found dead four days after; small intestine was distended with creamy mucus; no commabacilli in it of any kind, only small and largish straight bacilli. Lungs inflamed. Blood of heart contains bacilli, differing in thickness and length.

All rats (5, 6, 7, 8) remained well, mice 17 and 18 remained well.

It follows, then, from these experiments, that mice 11 and 12 fed with intestinal mucus of mouse 1, and not containing any commabacilli, died; whereas the mice 17 and 18 fed with the intestinal mucus of mouse 3, *i.e.*, the one containing numbers of commabacilli, remained perfectly well.

That the mice 11 and 12 died in consequence of the feeding cannot be proved, the symptoms were more like those of some kind of septicaemia.

(*b*) Diluted heart's blood of mouse 3 with neutral saline solution, and inoculated with it subcutaneously two mice 13 and 14, and two rats 15 and 16.

[This blood contained numerous commabacilli of at least two varieties.] Both mice 13 and 14 were dead seven days after.

Showed no symptoms during life. On post mortem examination lungs were found inflamed, small intestine distended with mucus; heart's blood contained large numbers of minute vibrios, but quite unlike the commabacilli of mouse 3.

The vibrios look more like imperfect spirilla. Many of the vibrios contain one or more granules. Besides these there were present thickish and thin straight bacilli.

The intestinal mucus contained various kinds of straight bacilli, differing in length and thickness.

(*c*) The blood of mouse 12 was diluted with neutral saline solution, and inoculated with it subcutaneously two mice (19 and 20), and two rats (21 and 22). One of these was found dead three hours after, lungs much inflamed; the other was dying 24 hours after. In the heart's blood were a few thickish bacilli; slight congestion of the intestine, lungs inflamed.

It follows from this that the death of these mice could not have been caused by the inoculation, since only a drop of the diluted blood was used for inoculation, and the state of the lungs in both animals was clearly of older standing than the inoculation.

The two rats remained well.

From these experiments it follows that the mice died under anatomical lesions, indicating some kind of septicaemia, and that death could not have been either the result of feeding or inoculation, since other mice died with the same lesions, but which had not been the subject of experiment.

The death of mouse 19 proves this beyond doubt, since it died with the same symptoms, and must have been suffering from the disease before inoculation was performed.

In some of the animals the small intestine was distended with a creamy fluid, containing numerous detached epithelial cells, and various kinds of bacilli. In one case several (at least two) kinds of commabacilli.

It is important to take notice of this fact, since there appears—judging from the statements of various French observers—a tendency to consider such an occurrence in animals experimented on with choleraic material as indicating that the animal had died of cholera. Nothing would be more unjustified.

Inoculation-experiments made with Cultivations of Commabacilli and the small straight Bacilli.

(*a*) With a pure cultivation of commabacilli started from mucous flakes of an acute typical case inoculated* two rabbits, into the subcutaneous tissue of the thigh. No result.

(*b*) With same cultivation inoculated two rabbits, into the peritoneal cavity. No result.

(*c*) With the same cultivation injected into the jugular vein of two rabbits. No result.

---

* In all these and the following inoculation experiments relatively large quantities were used for inoculation, *i.e.*, several divisions of a hypodermic syringe of the cultivation teeming with the commabacilli.

(*d*) A hypodermic syringe filled from this cultivation injected into the small intestine of one rabbit. No result. On dissection being made before the end of a week, all organs were found perfectly healthy. In the intestine no trace of any change, no commabacilli.

(*e*) With a cultivation on linen of commabacilli and the small bacilli, 24 hours old, inoculated two rabbits subcutaneously. No result.

(*f*) With same material inoculated two rabbits into the peritoneal cavity. No result. (One of these animals died on the eighth day with severe peritonitis, much peritoneal exudation and lymph. No commabacilli or any other organisms in the exudation or blood.)

(*g*) With same cultivation inoculated one rabbit into jugular vein. This animal was rather in a poor condition, having had an abscess on the back, due to caries of a dorsal vertebra. The animal died on the seventh day with severe peritonitis and pleuritis, copious sanguineous exudation, inflammation of the lungs; no commabacilli or any other organisms in blood and exudation.

(*h*) Of a mixture of a cultivation of commabacilli with a cultivation of small bacilli inoculated (injected one hypodermic syringefull) into the cavity of the small intestine of one small monkey. No result.

(*i*) Of same cultivation, injected about one half syringefull into the jugular vein of another small monkey. No result.

(*j*) Mucus flakes and fluid taken from the ileum of an acute typical case had been kept in a capsule for three days; after this time there were present in the fluid large numbers of putrefactive organisms, large bacilli and micrococci, in some of the flakes the commabacilli were present in enormous numbers, besides these there were numerous S-shaped and spirillum-shaped organisms, which could be distinctly seen to be derived from the commabacilli (see Fig. 13). Of these flakes full of commas injected one syringefull into the cavity of the small intestine of one cat. No result.

(*k*) Of same material injected one syringefull into the jugular vein of another cat. No result.

(*l*) Of a mixture of a cultivation of commabacilli and of small bacilli injected one syringefull into the cavity of the small intestine of one (third) small monkey. No result.

(*m*) Of same cultivations injected half a syringe into the jugular vein of one (fourth) small monkey. No result.

(*n*) Of same material injected one syringefull into the duodenum of a rabbit. No result.

(*o*) Ditto one rabbit into small intestine. No result.

(*p*) Ditto one rabbit into jugular vein. No result.

From all these experiments it follows that neither with mucus flakes taken from the ileum of acute cases of cholera, nor with stools recent and old, nor with cultivations of commabacilli or small bacilli, is it possible to produce in animals (mice, rats, cats, rabbits, and monkeys) any illness, be the introduction into the system carried out by feeding, by subcutaneous injection into the jugular vein, or by injection into the cavity of the intestine.

That the animals operated upon by Nicati and Rietsch and by Koch and others (mentioned on a previous page) did not die of cholera, but in consequence of the operation, must be evident to every one who has much experimented in Europe.

While cats readily get over abdominal operations, dogs do not do so, besides the fact that in the case of cats and monkeys, although considerable quantities of mucus full of commabacilli and pure cultivations of commabacilli were injected into the bowels, the animals did not die, and showed no symptoms, proves that the choleraic commabacilli are quite harmless on these animals; and if any animal would be likely to take cholera it would be monkeys; nor is it likely that there should exist such a vast difference in susceptibility between cats and dogs.

[Koch seems to think that the injection must be made into the duodenum, but how is this reconcilable with his statement that the disease has its seat chiefly in the lower part of the ileum ?]

It is well known that in India even severe surgical operations succeed much easier than in Europe, and this presumably applies also to animals; such severe operations as abdominal operations (opening the peritoneum and drawing out a loop of intestine) succeed readily, as has been shown, in India, on rabbits, cats, and monkeys; but

the experiments have been repeated in Calcutta on dogs with the same negative result.

In two dogs, the peritoneum was opened in the linea alba, and a considerable quantity of pure cultivation of commabacilli, derived from the mucus flakes of a choleraic ileum, was injected into the duodenum, in a third dog only water was injected; all these animals remained alive and showed absolutely no symptoms. From these experiments it follows, (1) that the animals operated upon by Nicati and Rietsch and by Koch did not die of cholera, but in consequence of the operation, and (2) that neither the commabacilli of cultivations nor those in the original mucus flakes of choleraic intestine are capable of producing cholera when injected into the small intestine of rabbits, cats, monkeys, or dogs.

Addendum.—Professor Horsley, of the Brown Institution, London, has made for Mr. G. Dowdeswell similar experiments on four rabbits, two guinea-pigs, and one dog with pure cultivations of choleraic commabacilli (derived from cholera cases in France as well as from India); a small quantity was injected into the upper part of the small intestine. *The operation was made antiseptically. All seven animals remained well.* Professor Horsley has also kindly made for one of us the following experiments on guinea-pigs and dogs.

Three dogs were operated upon antiseptically; of pure cultivation of living and fresh choleraic commabacilli in gelatine half a hypodermic syringefull was injected into the commencement of the duodenum of each animal. No result.

In a further series, five dogs were similarly operated upon, and commabacilli were injected into the duodenum, but no result followed.

Three guinea-pigs were operated upon, *not antiseptically;* of a cultivation of bacillus subtilis grown in gelatine and derived originally some 15 months ago from a Jequirity infusion—but passed through several successive cultivations in broth and proved to be quite innocuous—less than half a hypodermic syringe was injected into the commencement of the duodenum of each animal.

All these animals were dead before three days were over. The small intestine was much congested, its cavity was filled with mucus containing large masses of detached epithelium cells.

It must be clear from these experiments that the choleraic commabacilli injected into the intestine of rodents and carnivorous animals in London have neither the power of producing cholera, nor have they the power of producing any diseased condition, and therefore are not pathogenic organisms.

The assumption that the commabacilli introduced into the small intestine of animals are capable of multiplication, and of producing a special poison which, absorbed into the system produces death, is not borne out by these experiments. The fact observed by Koch and others, that the commabacilli are capable of growing and multiplying in the cavity of the alimentary canal does not prove anything for their assumed pathogenic property, since they would naturally be capable of growing in that locality which is their natural breeding ground. The deaths of the animals, then, operated upon by Koch and others, is not due to any specific disease-producing power of the commabacilli, but is due to septic infection or in consequence of the operation. The operation alone, exposure of the peritoneal cavity, the handling of the duodenum not performed under spray—leaving out any subsequent injection of the commabacilli—produces death with the same symptons as in the experiments of Koch and others. That the commabacilli, like other putrefactive bacteria, are capable of producing the septic poison (ptomaines) seems to appear from the later experiments of Nicati and Rietsch, who injected comparatively large doses of broth in which the commabacilli had been growing and from which, subsequently, the commabacilli were removed by filtration. The symptoms under which the animals died were identical with those of septic intoxication, such as are produced by the injection of putrid fluids from which the putrefactive bacteria had been previously removed. Nicati and Rietsch seem to be, strange to say, altogether unacquainted with the observations of Gaspard, Panum, Bergmann, Weber, Billroth, and many others who have studied and described the pathology of septic infection in dogs; the two French experimenters consider, stranger still, their own result as indicating the production by the commabacilli of a specific virus. If it should be said that the experiments performed by Koch, Nicati, and Rietsch, if they do not prove that cholera is transmissible to animals, at any rate prove that the choleraic commabacilli are capable of growing in the mucus membrane of the small intestine of animals and then to produce a chemical poison, hence are pathogenic,—the answer to this would be this: amongst the innumerable bacteria existing in normal human fæcal matter, there is

one species of minute bacilli which Bienstock has succeeded in isolating by artificial cultivation in alkaline solid media. These bacilli inoculated into mice produce death in 24 hours, bacilli being found in the blood of the animals (Centralbl. f. d. Med. Wiss. 1883, p. 949) ; and further : Pasteur and Sternberg have isolated from the normal human saliva a species of bacterium which produces fatal disease in rabbits.

In conclusion, we wish to render our best thanks to the following gentlemen, who, during our stay in India, have generously and liberally given us every possible assistance, and have secured to us every facility for carrying out our investigations:—

Dr. Cuningham, Sanitary Commissioner with the Government of India.
Dr. Barclay, Secretary to the Sanitary Commissioner.
Dr. Hewlett, Deputy Surgeon-General with the Government of Bombay.
Dr. Cook, Principal of the Grant Medical College in Bombay.
Dr. Waters, Pathologist in the same College.
Dr. Lyon, Professor of Chemistry in the same College.
Dr. Anna, Physician in the Cholera Ward of the same College.
Dr. Coates, Principal of the Medical College in Calcutta.
Dr. Waddell, Professor of Chemistry in the same College.
Dr. C. Mackenzie; and last, but not least, Dr. D. D. Cunningham, Professor of Physiology in the Medical College in Calcutta.

To Mr. Alfred Lingard, who has rendered most valuable assistance in every part of the inquiry, our special thanks are due.

E. Klein.
Heneage Gibbes.

APPENDICES.

Appendix A.

On the Relation of Bacteria to Asiatic Cholera.

In order to understand the exact relation of organisms to the cause of disease, the following circumstances have to be well borne in mind:—

(1.) A disease may be caused by a virus which is the product of an organism, but the disease is not a communicable one, or with other words the disease is not an infectious disease. An example of this kind we find in septic or putrid intoxication.

As is now well known through the researches of Gaspard, Panum, Bergmann, Sanderson, and others, certain products of putrefaction, resulting from the activity of the putrefactive bacteria on nitrogenous compounds, outside the animal system or within, when introduced in small quantities into an animal or man, produce a transitory pyrexia, and if the quantity of the material is sufficiently large, in addition, symptoms of acute poisoning, as vomiting, purging, hæmorrhage in the various serous and parenchymatous organs, collapse and death.

In these instances the poison itself can be and has been isolated and separated from the putrefactive organisms which produce it.

The blood and tissues of an animal thus poisoned do not while fresh contain organisms, and possess no infective properties.

Koch describes the nature and action of the cholera virus in a similar manner, viz., it is a product of (not putrefactive but specific) organisms, *i.e.*, the commabacilli. The poison itself, as in the case of putrid intoxication, is a chemical, *i.e.*, not organised, ferment produced by the commabacilli, which, having found entrance into the alimentary canal, multiply rapidly and produce the ferment which absorbed into the system induces the whole chain of symptoms constituting cholera. Just as in the case of putrid intoxication the directly active virus requires for its production the putrefactive bacteria, so also in the case of cholera, the cholera virus presupposes the existence and multiplication of the commabacilli. The essential difference between the two cases is this, that in the putrid intoxication, the putrid poison, as is evident from the nature of things, can be produced outside the body, and can be isolated from the organisms; whereas in cholera the commabacilli can only unfold their poisonous activity within the alimentary canal, *i.e.*, in the system itself. As has been shown on former pages, these inferences of Koch as to the relation of the cholera process to the commabacilli are based on propositions which have not been proved, and therefore cannot be accepted, and besides they necessitate the assumption of direct contagion, which is in opposition to all that is known of cholera.

(2.) Or a disease may be caused by a poison which is produced under certain conditions, but always outside the animal body, say in a suitable soil, and when finding access into the body of a person and without reproducing itself here sets up a definite group of symptoms. This poison may be the outcome of fermentative processes caused by an organism or not, *i.e.*, by peculiar chemical ferments. Thus in malarial and other fevers the virus does not reproduce itself within the body, it is produced outside the human body in a peculiar soil. Whether it is produced by the agency of organisms or by purely chemical fermentative processes is a matter of detail to be discussed below. At any rate this group comprises non-infectious diseases allied to those of the former category.

(3.) Or a disease may be an infectious disease, and caused by an organism in the true sense of the word.

This class comprises diseases which have this great and essential character in common, that they are directly transmissible; in their process of transmission there is always only an almost infinitesimal quantity of virus required to start the disease; that in every infected individual this virus multiplies to an enormous extent, so that all affected tissues and sometimes the blood teem with the virus. And it is owing to this fact of self-multiplication that it appears *à priori* probable that the virus in infectious diseases is a living entity.

In anthrax, glanders, tubercolosis, erysipelas, swine fever, fowl cholera, various kinds of septicaemia, &c., this entity has been definitely identified, isolated, cultivated, and with it the disease reproduced. In all these instances it has been found to be an organism belonging to the tribe of bacteria (micrococcus, bacterium, and bacillus), and in all these instances the same kind of organism has been found in the blood or diseased tissues of the individual infected either with the artificial cultivation or the original material. In other infectious diseases this whole chain of evidence is incomplete; in some, *e.g.*, typhoid fever, small-pox, syphilis, the organism has not been clearly demonstrated. In still other infectious diseases the organism has been identified, but not isolated, and its activity has not been tested, *e.g.*, relapsing fever. In a last group of infectious diseases it has been identified and isolated, but owing to the unsusceptibility of animals to the disease, its activity cannot be tested, such is the case in leprosy.

(4.) Or a disease might be due to a virus which, although undergoing multiplication within the body of a person affected with the malady, does not leave the body as actual virus, but requires some intermediate stage to become so. An instance of this kind is not known.

A disease of this kind would be an infectious disease, but not in exactly the same sense as one mentioned Sub. 3.

Now the question arises, in what category of diseases should cholera be placed? The first point, and one which is obviously of the greatest importance, is this,—is cholera an infectious malady in the true sense of the word, or is it not?

Is cholera a disease of which it can be said that the virus introduced into a suitable individual increases to an enormous extent, and thus yields a crop of new virus, the smallest quantity of which when finding access to a new individual is capable of starting the malady, like anthrax or small-pox?

As is well known from the immense literature of the subject, there are a good many observers who, on account of their Indian experience, question the correctness of the view that cholera can be communicated from individual to individual, either directly or indirectly, *i.e.*, through human intercourse, but they maintain that cholera, like malarial and other fevers, is independent of human intercourse, but owes its origin to peculiar fermentative products of the soil. According to this view, the doctrine of contagium vivum does not apply to cholera.

Now, this view as to the independence of cholera on human intercourse must and does appear to medical men in Europe very strange, considering that the history of almost every visitation of European countries by cholera clearly points to the invasion of the disease by human intercourse. But when one comes to consider the conditions obtaining in India, such a view as above stated to be held by very experienced observers in India loses all its strangeness.

One of the best established facts in connection with cholera amongst Anglo-Indian troops in India, and on the strength of which invariably successful action is taken, is this, that as soon as in any cantonment cholera appears amongst the soldiers, the troops, including those affected, are removed to camp, and cholera ceases.

If cholera were communicable directly or indirectly from one individual to another, why, one might ask, should it cease under these new conditions? since in many instances cases contracted in the cantonment, but coming to head only after removal into the new camp, are still there to sow out the virus? The habits of the soldiers remain the same, the evacuations are disposed of in the same way as previously, and nevertheless cholera does not spread. Or take the conditions obtaining amongst the Native populations. Benares, as is well known, is a centre in which continually large numbers of pilgrims congregate.

The sanitary conditions prevailing in this, as in other cities, are so primitive, that, seeing and to a great extent smelling the insanitary state, one is lost in amazement how it is possible if this theory be correct that cholera is not constantly there epidemically. The Ganges, on the shores of which the city is located, is considered by the Hindoos as the sacred river, and therefore ablutions in it by the pilgrims and natives of the town are carried on to an enormous extent.

But not only external ablutions, internal ablutions as well, as also washing of garments and linen, are carried on on a large scale.

It is a curious sight to see hundreds of Hindoos bathing in, washing their mouths and teeth with, as well as drinking the water of these filthiest of waters. One of the main drains of the town discharges its filth into the river at a place close to the nest of temples. The number of people bathing here, washing their mouths and teeth,

washing their clothes, drinking, carrying away the water in vessels for domestic consumption, at this very spot, in tangible proximity to this unsightliest outlet, is simply extraordinary.

The drainage of the city is so defective that in every street the stagnation in the drains is a thing that obtrudes itself in a very unpleasant manner to the eye, and still more to the nose, and this finds almost its climax in the very vicinity of the temples and shrines, where constantly processions of pilgrims pass up and down. The domestic arrangements are of the most primitive nature. Some sort of latrines are found even in the houses of the poorest, but these latrines communicate only with defective and, in many instances, stagnant drains, and, besides, a contamination by human dejecta of the house wells is not only not excluded, but is, one might almost say, the rule. And this is not only the case in the poorest houses, but, as direct inspection proved, in well-to-do establishments.

Even the public wells, of which there are a great many in the town, and the water of which is ostensibly used for drinking purposes, are such as not to exclude constant contamination. They are surrounded by a raised platform of masonry, and on this platform there is washing of clothes carried out. Now in every well that was examined the masonry of the well itself was defective, and in several instances the fluid filth was distinctly seen oozing into the well through the defective masonry.

Now, if under these conditions a case of cholera occurs in any locality of the town, how, one might reasonably ask, is it possible that this can ever remain isolated? how is it that it is not followed by an epidemic outbreak of the disease? That the people of Benares have not become unsusceptible to the disease is proved by the fact that epidemics do occur occasionally. In the latter half of October, that is, some time after the rains, Benares had only a few isolated cases, although the sanitary conditions are such that an epidemic then and at all times would have been the very thing to be expected.

Or take the conditions obtaining in Bombay. In two quarters in Bombay, Dobie Talao and Cavel, there occurred a good many cases of cholera during the month of September.

In both there is an attempt at drainage, but, owing to the habits of the people of using the street gutters instead, any attempt of collecting and guarding human excreta must be futile. Not only is this the case, but the excreta in some streets (owing to the heavy rains) find their way from the water-closets at the back of the houses into the streets, and here the liquid sewage can be seen and smelt spreading in the gutters and all round them. In addition to this, there are wells situated in the street, or in some instances, *e.g.*, in Dobie Talao, at the back of the houses. In both instances the passage of the liquid sewage into the wells through the defective masonry can be detected without any difficulty.

Although the people are not supposed to drink from this water, using it ostensibly only for washing of clothes and domestic utensils, it is nevertheless a well known fact that sometimes they do use it, since the water supply (from the Vihar) is an intermittent one, and in case of the deficiency of this drinking water (which occurs during the daytime), the people do not hesitate to use the well water for drinking purposes. On first questioning them they do not, of course, admit it, but on cross-examination it has been invariably brought out that they did and do use it so.

In addition to all this, one must bear in mind that their cooking utensils must be constantly contaminated with filth and filthy water, and to all this add the habits of the Natives of eating with their bare and by no means clean fingers, and one can easily understand that they embody a considerable amount of filth in the shortest time.

In an alley in Cavel, in which lived about 50 people, each family in a small room leading out into a common court, there occurred in one of the rooms two cases of cholera, two brothers, mendicant priests. The sanitary conditions here were the same, or worse if anything, the people all being very poor, and the habits of these brothers in the way of disposing of their filth differed in no way from the general rule, and nevertheless no more cases of cholera occurred here. The sanitary conditions existing in other parts of the town, and particularly in villages, are simply described by the word *nil*, and nevertheless a case of cholera occurs occasionally, but does not spread. The disposal of the excrements, the washing of clothes, linen, and utensils in the water (tanks) used for general and drinking purposes, all their habits, their eating with unclean hands or after washing them with the filthy water, are the same.

In Calcutta the conditions amongst the Natives, in the groups of huts (bustees) interspersed amongst European houses, and often situated around a tank, are the same everywhere; tanks, supposed to be only ornamental or for drinking purposes, are

nevertheless, as may be seen almost everywhere, used by the Natives for their external and internal ablutions, for washing their clothes, and even for more unsightly though natural purposes, and the Natives often drink of this water.

How is it then, one might reasonably ask, that if once a case of cholera occurs in one of the houses around such a tank, it does not spread? That at certain seasons of the year (during the months of January, February, and March) it assumes considerable and sometimes alarming proportions, is no answer to this question, since during the hot season, when it ought to spread, if the contagionist theory pure and simple were true, it ought to be more prevalent, whereas just the contrary is the case.

Now, do not all these facts support in a conspicuous degree the view held by many experienced observers, that cholera is not directly communicable from the sick to the healthy,— that it is dependent in the first place on locality and season?

What, in the face of such facts, which could be multiplied many times, and had been done so in a most clear and thorough manner by Bryden, v. Pettenkofer in all his writings, from experiences in India and Europe (*e.g.*, the immunity from cholera experienced by Versailles, Lyons, and other cities), becomes of the view of the contagionists, who, like Virchow, Koch, and others, must of necessity maintain the possibility of direct contagion, if the commabacillus is to be of any account in the matter? The perfectly gratuitous statements of Koch (*l. c.*) as to the ready and direct transmission of the disease by linen soiled with evacuation of cholera patients, the still more gratuitous assertions as to the spread of cholera on board ship from patient to patient, lose all significance when compared with the abundant and overwhelming evidence as to the non-communicability of the disease directly from the sick to the healthy, and as to its dependence on locality and season.

Even granting for the sake of argument that Koch's statements with regard to the distribution of the commabacilli are correct (statements which have been shown previously to be in flagrant opposition to the facts observed by us), his inferences that the commabacillus is the cause of cholera cannot be true, since cholera is in an eminent degree a malady which is not directly communicable from the sick to the healthy, but which it ought to be under all conditions if it were dependent on the commabacilli, seeing that these are voided by the sick in enormous numbers, and that they, in India at any rate, find constantly and copiously access to the system of persons. If additional proof were required to bear this out, the experience in the Hospital of the Medical College in Calcutta where Koch worked, and as is well known in other hospitals in India as well as in Europe, might have furnished Koch with it. Here cholera patients are put more or less indiscriminately amongst other patients, and still neither attendants nor other patients ever contract the disease.

Every one accustomed to work with bacteria knows well how insignificant and useless ordinary precautions of cleanliness are of keeping bacteria out from where one does not want them, and the commabacilli would make no difference from this.

True, Koch says that, the commabacilli being killed by drying, particles of the evacuations, although at first full of living commabacilli, would, by drying on linen, on the floor, &c., become non-infectious. Now, as is well known, micrococci and bacterium termo are also killed by drying, and as these have no spores, *i.e.*, permanent seeds, contamination of culture media by them simply through the air should be impossible, and nevertheless, as is known to every worker in this field, the contrary is the case. This simply proves that the micrococci and bacterium contained in minute particles of putrid substances wafted about by air currents are not really dry, since, if they were so, they would be dead, and could not produce contamination.

That in a ward where a number of cholera patients are constantly soiling the floor and beds with their evacuations, and where the attendants are constantly handling these, it is absolutely inconceivable that no contamination of their persons should occur with the organisms present in the evacuations, and amongst others also with the commabacilli; to say that probably the attendants are all in robust health, and therefore insusceptible, does not meet the case, because some of the attendants are not so, and besides, what of the other patients in the wards? A visit at meal times, seeing sickly and weak patients eating with their fingers, would soon convince everybody of the absurdity of such a view.

Another point which Koch adduces to explain the non-communicability of cholera directly from the sick to the healthy is this. Koch says that the commabacilli cannot live in acid medium, and therefore, when taken into the stomach of a healthy person, they cannot pass unscathed into the small intestine, their breeding ground. But this assertion of Koch's as to the fatal influence of acid on the commabacilli is not borne

out by observation; on the contrary, the commabacilli are not killed by weak acid, as has been shown on a former page.

The writings of v. Pettenkofer have fully and satisfactorily explained those few cases of transmission of cholera by linen, not because of their being soiled with cholera evacuations, but because they are derived from an infected locality. In those few instances where, on ships and in a hospital, apparently, a spread of the disease has been observed, this apparent spread has been caused, not because a cholera case was there, but because the ship or hospital had become itself a cholera locality, and it is not necessary to enter here further into this. If anything can be made of these instances, it is certainly not in favour of the contagionists.

There is then no reason to suppose that the discovery of the commabacillus nor the small straight bacilli above described supplies the contagionists with new facts by which to support their theory, because in the first instance all the known facts as to the spread and distribution of cholera are against such a theory, and, in the second place, because the statements as to the relation of the commabacillus to the disease are not sufficiently established. But it might be argued, if the commabacillus or the small straight bacillus are not the cause of cholera, then some other organism must be it, since it is said with great accentuation that cholera can be spread by human intercourse; it is further assumed that the cholera poison or something that can develop into it, having found entrance into an individual, multiplies there, is discharged by the evacuations, finds access to a peculiar favourable locality, and then supposing other conditions favourable develops into the real poison, which having had entrance in some way or another into a healthy person creates the disease. That the virus is not present as such in the cholera evacuations is clear from the fact that cholera is not directly communicable; that cholera evacuations when brought into a suitable soil ever develop the virus there is no direct evidence to show, the presumption is great, but as regards direct proof there is none.

The importation of cholera into Europe and the Mediterranean countries, as is well known, cannot be traced in all cases to the importation of a person affected with the disease, and, in some well established instances (Hirsch, Pettenkofer, Sander, and others) has been traced to the importation of a something—articles of linen or clothing—derived from an infected locality, but which articles had not been soiled directly by cholera evacuations; see Hirsch, Berliner-Klin. Woch, N. 31, 1884.

While then on the one hand there is direct evidence to show that the cholera evacuations do not and cannot contain the active virus, there is also this evidence that organisms like the commabacilli and the small straight bacilli, constantly occuring in the mucous flakes, can have nothing definite to do with the disease. But no micrococcus, bacterium, or bacillus, or any other kind of bacterium, fulfils the elementary conditions just expressed.

One might say, from the knowledge that bacilli have the power to produce spores, that it might be a bacillus. The bacillus itself as it passes out of the body of a patient might be inactive, but its spores formed outside the body, in peculiar soil and at certain seasons, might be the active principle. Those who would accept such a view would, of course, say that the virus is a bacillus which can unfold its poisonous activity only when introduced into the new individual as spores, the fresh evacuations containing only the bacilli are inactive. Allowing, for the sake of argument, this, a simple consideration will show its unteneability. These hypothetical spores introduced into the body of a person would germinate into bacilli, and these would then multiply by fission after the manner of bacilli, and hereby produce the chemical ferment necessary to start the disease. Spores of anthrax bacilli introduced into a suitable animal germinate into the bacilli, these multiply to a great extent, and cause the disease known as malignant anthrax. And this simile might be further carried by saying that, just as is the case with the anthrax bacilli, they do not form spores unless certain conditions, as temperature, moisture, and free access of air, so also the hypothetical bacilli vacuated by a cholera patient, would produce spores only when placed under the necessary conditions. But this simile suffers from a cardinal defect; it is this,—in anthrax, the bacilli are as virulent as the spores, and there is no case known of bacilli, septic or specific, in which the bacilli themselves have not precisely the same function as their spores.

[It might be said that, as is the case in some conditions in anthrax, the bacilli may be innocuous, whereas their spores may be poisonous, *e.g.*, bacilli taken from the blood of an animal dead of anthrax, when grown at high temperatures (42 to 43 C.) for sufficiently long time (two to three weeks) may lose their activity entirely on sheep, whereas if the bacilli are used when growing at ordinary temperature or when allowed to form

spores prove active on sheep. And it might be said that a similar thing may be the case in cholera, *i.e.*, the hypothetical bacilli of the evacuations might be inactive, but when growing under certain conditions (of soil and season) may prove active either as bacilli or after having formed spores. But it must be evident that the cases are widely different from one another. The comparison could be applied with a certain semblance of feasibility, if the anthrax bacilli produced in one animal and used fresh proved inactive on the same species, but acquired potency when grown under conditions different from those obtaining in the animal body. As far as our knowledge goes, just the contrary is the case with all pathogenic bacteria, they being potent when fresh, *i.e.*, directly taken from the infected animal.]

The strongest reason for not admitting this kind of bacillar relation to the disease is this, that no bacilli exist in the blood or any other tissues of patients suffering from cholera.

There is absolutely nothing that in the remotest degree could bear out such a bacillar origin.

The blood and other tissues, intestines, mesenteric glands, liver, spleen, kidneys, lungs, spinal cord, of acute typical cases have been carefully examined after the approved modern methods, and nothing could be found of bacilli, except occasionally, but comparatively rarely, a few bacilli, clearly of putrefactive origin, could be seen in the portal vessels of the liver, in the dead portions of the mucosa of the stomach, and in the cavity of the Lieberkühn's follicles, as well as in the spaces between the detached epithelial lining and the membrana propria of these glands, but this only rarely and in very few places, and when it occurred it was clearly due to post mortem changes. Cholera disease is so rapid in its development and course, its symptoms are so complex, and so many different nerve-centres, as the centres of circulation and respiration, secretion, the alimentary canal and muscular system, are affected, that there can be no doubt that the actual virus must have found entrance into the blood and circulation, and thence is distributed to and acts on the various centres. Koch, not finding any commabacilli anywhere except the intestines, overcomes the difficulty by saying that the actual virus is a chemical ferment, produced by the commabacilli in the intestines, and its absorption into the system sets up the symptoms of the disease. But since the assertions on which this theory is based are not sufficiently supported, *i.e.*, the assertion as to the presence of commabacilli in the living tissue of the intestine, this theory cannot be accepted. For the same reason the small straight bacilli can have nothing to do with the disease, and as there are no other organisms present, it follows that *the body of a patient suffering from cholera contains no organisms of any kind that can be associated with the disease.*

From all this it appears that there is no evidence obtainable that the disease is associated with a distinct specific form of bacteria, a fact which was expected from reasons stated above.

The *a priori* probabilities of there being a micro-organism connected with the disease belonging to a higher order than bacteria (*e.g.*, hyphomycetes, &c.) are not much greater than for the bacteria, for although they often show very complicated changes in their process of growth and development,—witness the complexity of the growth and development of a penicillium, aspergillus, mucor, saprolegnia, &c.—it is an established fact that, in all these instances where the mycelium succeeds in growing and thriving, also the spores succeed in germinating and giving again origin to the mycelium. It might be said that it requires the spores to set up the disease, for they can make a start where the mycelium might be incapable to do so, and this is to a certain extent borne out by the observations of Grohe, Lichtheim, and others, who have shown that, after the introduction of the spores of certain species of aspergillus into the rabbit, a general mycosis can be produced, and concluding from this it might be argued that, supposing cholera to be due to a hyphomycetes, and supposing also it were necessary that the mycelium should be first brought into a proper soil and at proper time,—and thus satisfying the theory of the localists,—in order to be capable of forming spores, which, when finding access into the human body, set up the disease cholera.

This may be possible, but it is not probable, for the simple reason that in cholera there is no part of the body where anything of a spore or mycelium can be detected. [Assertions have been made that there have been seen in rice-water stools that had been kept for four days organisms, in the shape of a kind of mucor, which form cysts, which again give origin to spores, which again give origin to a mycelium, which again produces cysts, which ultimately yield a crop of commabacilli; but all this, on account of its inherent improbabilities, the coarseness and superficiality of the method

of investigation, is so absurd, and is so much at variance with what is known of these microphytes, that it neither requires nor deserves serious consideration.]

Thus the conclusion is forced on us that in cholera no micro-organism that can be in any way connected with the disease is present in the body of a person affected with cholera.

How then is it possible to explain the invasion by, and spread in a locality of the disease? No doubt the assumption of a specific living organism, which, in one form or other, having gained entrance into a person, there multiplies and produces the disease, and then is passed out in enormous numbers into soil suitable for the peculiar change, in the course of which it assumes its potency, is a feasible one, and at the same time simple, and it is one towards which, in some of his later writings at any rate, v. Pettenkofer inclines, who as yet has given the most satisfactory view of the mode of propagation and causation of the disease, as contrasted with the view of the contagionists, who assume an organism capable of starting the disease as it passes out of an infected body.

As stated just now, the assumption of a micro-organism passing out of a choleraic patient being indirectly connected with the disease, *i.e.*, by and by acquires potency, is very simple, but it is not absolutely necessary, and as has been shown is contrary to the facts observed.

Supposing somebody maintained that the choleraic evacuations contained the virus in a potential state; as has been shown above this cannot be an organism, since the organisms present in the evacuations do not fulfil the elementary conditions required of the choleraic virus, and therefore he would have to assume that it is a chemical ferment. But against such a view there is this great difficulty, that this potential virus must be capable of self-multiplication, for introducing an infinitesimal quantity of it from a cholera locality into a new place it is capable of producing an epidemic outbreak of cholera. A self-multiplying thing cannot be simply a chemical ferment, according to all our notions this must be a living organism.

In the face of the fact that no micro-organism hitherto proved as connected with infectious diseases would fulfil all the conditions required of the cholera virus, and detailed on a previous page, and in view of the fact that no micro-organism can be found in the body of a person affected with cholera, as being connected with the process of the disease, it is impossible to maintain that the evacautions of a person affected with cholera contain actually or potentially the cholera poison in the shape of an organism, and from this it follows that the direct cause of cholera is not an organized ferment which has found entrance into the body of the patient, which there multiplies and passes out again in enormously increased numbers, but it must be some kind of ferment produced altogether outside and independently of the body of a cholera patient.

There can be no question that a cholera case introduced into a suitable locality can and does transform this locality into a centre of the plague, and there can be likewise no doubt that cholera can be and is occasionally introduced, not by a patient at all, but by some articles coming from an infected locality, Pettenkofer,* in his various writings on this disease, has very clearly drawn attention to all these facts, and it is not necessary to enter here further into them. Some very remarkable instances of very rapid infection (half an hour to a few hours) through linen soiled with choleraic evacuations, and coming from a cholera locality, are given by Professor Drasche, of Vienna. ("Der Pilzfund in der Cholera," Vienna, 1884, pp. 14. 15.)

The cholera virus producing an epidemic is then created in this new suitable locality by something transferred to it from an infected centre. This something must obviously be self-multiplying, and as no chemical ferment fulfils this elementary condition, it is necessary to assume that this something is a living entity, an organism. And, as was stated above, the direct virus being a chemical or non-organized ferment, we arrive at the conclusion that a living organism transferred from a cholera locality into a new and suitable soil, therein multiplies and gives rise to the production of a chemical ferment, which, when finding access to the body of a person, sets up the disease cholera.

The alkaloids known as ptomaines, produced by micro-organisms outside the animal body, and acting as deadly poison to the animal economy, may be quoted as illustration.

---

* That the choleraic evacuations *per se* do not contain the virus is a conclusion in harmony with all the facts known of the disease, for otherwise the disease would be directly contagious, which, as has been shown, it eminently is not. Pettenkofer, in his little pamphlet "Die Entdeckung des Cholerapilzes, Munchen, 1884," gives on p. 8 and *passim* a very remarkable instance of an epidemic, that of the prison Laufen, in which the harmlessness of choleraic evacuations, recent and old, is well illustrated.

Exception might be taken to this theory, on account of the difficulty of explaining the incubation after infection with a chemical poison. But the difficulty is far less than at first appears. The chemical poison, present in the seeds of abrus precatorius or Jequirity—the abrin of Messrs. Waddell and Warden—requires generally 24 hours to devolop its poisonous property, consisting in inflammation and œdema at the seat of inoculation, followed by death of the animals after two or three days.

It might be asked now, how is it possible to explain on this theory the well-known fact that linen soiled with choleraic evacuations have been capable of infecting with cholera persons who have handled these articles, unless by the theory that the evacuations after some time have devoloped the virus?

In the first place it is equally well known that such an infection is, on the whole, of rare occurrence, and one will ask in return, if the cholera evacuations are capable of developing the virus, how is it that this kind of infection is of rare occurrence?

Clearly because the evacuations alone are not capable to develop the virus, but in those cases in which they acquired this power something else had been added, some organism which was capable to develop the virus while growing in the evacuations. This leads us then to this theory. The cholera evacuations alone do not contain the virus either potentially or actually, but if something else, the extraneous specific organism, finds access to them this is capable to produce the virus in the evacuation. But this organism does not necessarily require cholera evacuation for its multiplication and for the creation of the virus since it is capable of doing this also in other suitable soil.

This theory, then, seems to be capable of explaining all the facts concerning the spread of cholera. To repeat it: the cholera evacuations, *per se*, do not contain actually or potentially, the organism which by its multiplication creates the cholera virus, a chemical ferment; this organism is extraneous to the body of an affected person; when transferred to a suitable locality or when finding access to the cholera evacuations or other filth is capable of multiplication, and of creating the chemical ferment, the actual virus.

Once having been created, the choleraic virus is introduced into the human body in various ways. A view favoured by many is the one that water is constantly the vehicle, others assume in addition the food, still others also the inspired air. There exist on record a good many cases in which water, as the vehicle of the virus, is put forward as having been proved, but the majority of these, on critical examination, do not stand. There can, on the other hand, be a good many instances quoted where water contaminated with choleraic evacuations can be proved to have been incapable of producing the disease. There is in India not a tank, not a pool, not a well in village or town to which, on the one hand, choleraic evacuations have not access, and from which, on the other, the Natives do not use the water for drinking purposes, and nevertheless, except in years of epidemics, isolated cases only are heard of around these tanks and wells. One might reasonably ask, if the cholera evacautions contained the virus, why does not one case of cholera at once produce a wholesale outbreak? Benares was mentioned on a former page as giving a good illustration, so does many a quarter in the native part of Bombay, so do many localities in Calcutta, and so do almost every city and village where a case of cholera does occur.

The cases, such as the noted Broad Street pump outbreak, where the water had been notoriously contaminated by sewage and choleraic evacuations, do not prove that these latter contained the virus, since, if cholera evacuations had access to the well, then also other matters of the soil may, and probably have, had access to it.

That water can be a vehicle, provided the virus formed in the soil has had access to the water, is, however, altogether a different matter. Such cases, well established, are on record, and amongst them the cases quoted in the Sanitary Reports for India, in those of the Local Government Board of London (the Broad Street pump epidemic), with reference to London water supply, are good instances. In order to satisfy the condition, that dilution of the virus by water would neutralize the action of the virus, if it were simply a chemical substance, it is necessary to assume, that the (chemical) virus is fixed on solid particles, possibly on the organisms that produce it.

And similarly, there is no reason to exclude food as a vehicle, provided it becomes contaminated with the material containing the virus either directly or through water. And likewise the possibility of the virus getting access to the body by means of the inspired air is perfectly admissible (see "Dryden Reports on Cholera in the Bengal Presidency from 1817 to 1872"), considering that, if the virus is created in the soil, it can escape the soil in various ways, and be inhaled by a human being just like the malarial poison, and, further, considering that the nature of the disease cholera is

such as to necessitate the assumption that the virus must have entered the blood in order to start all those complex symptoms, due to the disturbance of so widely different centres and organs. In addition to this, there are several well established instances known in which the handing of linen previously soiled with choleraic evacuations has been capable of producing cholera within as short a time as half an hour (see "Drasche: Der Pilzfund bei der Cholera," Vienna, 1884). In these instances clearly the virus must has been a chemical ferment that had found access to the organs of circulation within such a short time, which it could have done only by the respiratory organs.

E. Klein.
Heneage Gibbes.

Appendix B.

On the relation of Water contaminated with Comma Bacilli to Cholera.

The assumption by Koch that the commabacilli are connected with the cause of cholera has received a remarkable confirmation by Koch himself. As will be remembered, Koch, while in Calcutta, by one of his later reports to his Government, and particularly by an article that appeared in the "Englishman" of Calcutta, 18th February 1884, the substance of which article has been telegraphed to all European papers, has greatly startled all those that did not at once readily believe in the commabacilli as the cause of cholera. Koch therein states that cholera having broken out in one of the bustees surrounding a tank in a suburb of Calcutta, he visited this bustee and found numerous commabacilli in its tank. On a second visit, a week later, the epidemic being on the decline, he found much fewer commabacilli in that water, and this seemed to him and the "Englishman" to furnish positive and remarkable proof that these commabacilli were the cause of the cholera outbreak. It is known to all who have been in India, and has been mentioned on a former page, that the Natives use the water of every tank, ditch, and pool, however dirty and filthy, for all kinds of purposes,—bathing, washing of mouth, washing of domestic utensils, washing of clothes and linen, and even for drinking purposes.

This particular tank visited by Koch, like most other tanks, is surrounded by Native huts, and is used as a sort of common reservoir into which the evacuations of man and beast, and every kind of domestic filth, find access.

That the water of such a tank, around which cholera cases occur, and into which the evacuations of cholera patients find access, and in which the clothes soiled with cholera dejecta are washed, should contain the same commabacilli that are present in the choleraic evacuations is what one would naturally expect, and likewise that the number of these commabacilli should be fewer, the fewer the cholera cases, *i.e.*, the smaller the number of commabacilli thrown into the water. But to conclude, as Koch does, that because there are commabacilli in the water cholera cases occur amongst the people using the water, and as soon as the number of the commabacilli decreases in the water the number of cholera cases becomes less,* is manifestly illogical. That Koch should have used an argument of this nature to build up this theory is only intelligible if we remember how little convinced some of the medical public appeared to be of Koch's theory by his reports, and that it required, as it were, a much stronger argument to confound his critics. This discovery of the commabacillus in the water of that tank was considered such an argument, as is clear from the manner in which at the time the daily and some of the medical papers wrote about it.

That the cholera virus, whatever this is, can find entrance into a person by being conveyed there by water is in perfect harmony with the facts of the case, and that pure drinking water not contaminated with any extraneous material is of the greatest importance finds a very good illustration in the Reports of the Privy Council Office, in the Broad Street pump cases in London, and in the various Indian Sanitary Reports.

Another curious illustration how even a very experienced observer like Koch sometimes becomes unable to interpret correctly plain facts, is furnished in the same reports

* This last statement of Koch's requires a certain amount of correction. The tank of which Koch speaks was visited by him on 13th February, and again on 20th February. During the week he says the comma bacilli had greatly diminished, but in the records of the police office I find that the epidemic in the bustee surrounding this tank broke out on the 21st January, and lasted till 27th April. It lasted, therefore, fully two months more after this conspicuous diminution of the comma bacilli.

he sent to the German Government. Koch states that in Fort William, in Calcutta, cholera abated as soon as a good water supply to the fort was introduced, and takes this of course as proof that, previous to the introduction of the good water supply, many cholera cases were due to contaminated water. Now, had he taken the trouble, as he might easily have done, by looking at the records, he would have found that such a conclusion is quite out of harmony with the actual facts, for he would have convinced himself, by studying the records, that cholera cases diminished in a very marked degree some years *before* the introduction of the better water supply, and that this diminution, but no greater one, was kept up afterwards.

The Indian "Medical Gazette" of November 1884 republished, on page 332, the official statistics as to the course of cholera in Fort William, from 1856 to 1876. In 1863 there occurred a sudden decrease of cholera, and this decrease was kept up till 1876. But the new and pure municipal water supply was not introduced in 1862 or 1863, but in 1872, *i.e.*, nine years later than the conspicuous decrease of cholera.

We have had the opportunity in connection with Dr. D. D. Cunningham to make an examination of the water of some of the tanks in Calcutta, with reference to this very question of the commabacilli.

The same tank that plays such a conspicuous part in Koch's report above mentioned was visited on the 26th November. It is situated in Sahil Bagan, a suburb of Calcutta, and is marked on the subjoined Plan I. as Tank I.; it is surrounded by Native huts, in which about 200 families are living. There had occurred one case of cholera in this bustee about the first week of the month of November. The water of this tank was very dirty, particularly all along the shore, and the people around the tank, as is customary, made use of the water for all and every kind of domestic and other purposes, including drinking.

A sample of this water was taken from near the shore where it appeared particularly impure, about 20 yards from the house in which the cholera case had occurred, and the microscopic examination revealed undoubted commabacilli identical in every respect with those found in choleraic dejecta. Notwithstanding their presence in this water, and notwithstanding the extensive use the 200 families were constantly making of it, there has been no outbreak of cholera. Now we have in this instance an experiment performed by nature on a scale large enough to serve as an absolute and exact one. This water had been contaminated with choleraic evacuations, and of course with the commabacilli, and it was used extensively by so many human beings for several weeks; if, to speak with Koch, the commabacilli were the cause and essence of cholera, how is it that not one person amongst so many has, until the middle of December, contracted the disease? Clearly because the water did not contain the cholera virus, and because this latter has nothing to do with the commabacilli.

It might be said that perhaps the commabacilli present by the end of November were not the same as the cholera bacilli; but it must be remembered that there having occurred here a case of cholera, owing to the conditions obtaining here and owing to the habits of the people, large quantities of cholera bacilli must of necessity have been thrown and carried into this tank; along the shore the water contained abundance of decaying animal and vegetable nitrogenous material to form a very good and suitable nourishing medium for the bacilli, and they must have had ample opportunity to multiply, and consequently there must have become large numbers of them available sufficient for hnndreds of human beings. And nevertheless no case of cholera occurred.

And the same argument can be applied to most tanks and to most bustees in which once a case of cholera has occurred. The commabacilli of the choleraic evacuations find readily access to the water of some of the tanks, and there is absolutely no reason to suppose that they would not multiply, at any rate near the shores, where there is always present a good deal of decaying animal and vegetable matter, and contaminate the whole of the water of the tank, and consequently there ought to be an epidemic in the bustee surrounding such a tank. But this is manifestly not the case.

Close to this bustee is another bustee surrounding a tank, marked II. on the plan. Also in this bustee there are about 200 families. The water of this tank is as dirty as that of Tank I., and is used as extensively as the former. A sample of water taken from near the shore, and examined under the microscope, revealed, besides numerous bacillus subtilis, examples of undoubted commabacilli, in every respect identical with those found in Tank I. Amongst the 200 families living around this tank, and constantly using this water, there has not been a single case of cholera during the whole of 1884.

An equally striking illustration of the innocuousness of the commabacilli is furnished

by a tank situated near Teleepara Lane, in Calcutta (see accompanying Plan II.). Between the 14th and 16th of November there occurred nine cases of cholera in three houses of Teleepara Lane. In the accompanying plan the three houses are seen situated around a bend of the street, and they are Nos. 3, 4, and 34. No. 34 had three cases, No. 3 had three, and No. 4 had three cases. The people of No. 34 are rich Hindoos, and also those of No. 3 and No. 4 are well-to-do. Two of these three houses have their own hydrant, and from it they have a good supply of very clear water, such as is supplied to all good houses in the town. There is no condition common to all three houses, except that just in front of each of them there appears to be a communication with the street sewer. A narrow passage, marked XX., leads from Teleepara Lane to a bustee surrounding a large tank, marked III. on the plan.

As is usual, the people (low caste) living in this bustee make extensive use of the water of this tank, but the people of those three houses, being well-to-do and having their own drinking water, never went near this tank. In one of the huts of this bustee lives a milkman, who supplied, amongst others, house No. 34 of Teleepara Lane, but not No. 3 or No. 4. The water of Tank III., as usual, is very dirty, especially near the shore, and a sample of it examined under the microscope revealed the comma-bacilli. Amongst the people of this bustee there had not occurred a single case of cholera during the whole year.

It is quite clear from all this that the statement of Koch and his adherents as to the importance of the commabacilli in the water in producing cholera is in direct opposition to the above facts.

E. Klein.
Heneage Gibbes.

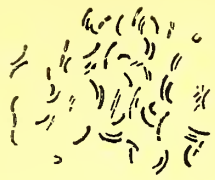


Fig. 1. From a preparation of fresh mucus flakes of a choleraic evacuation. Magnifying power about 700.
Showing large numbers of commabacilli, and a good many minute straight bacilli. Amongst the commabacilli there are a few small semicircular ones.

Fig. 2. From an artificial cultivation in Agar Agar jelly of the above commabacilli. M. p. about 700.

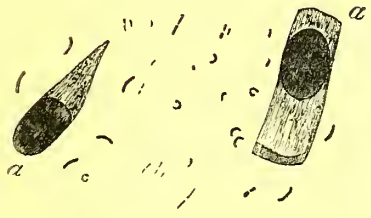


Fig. 3. From a preparation of fresh mucus flakes of the ileum of a typical rapidly fatal case of cholera. M. p. about 700.
(*a*) Epithelial cells.
Various kinds of bacteria are seen here; commabacilli of the ordinary type; small semicircular ones; minute straight ones; and somewhat large straight bacilli.

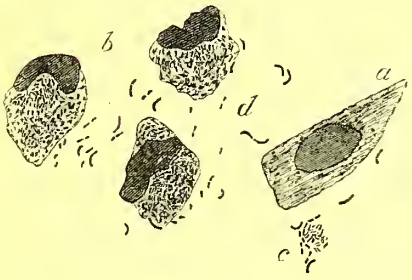


Fig. 4. From a preparation of fresh mucus flakes of the ileum of a typical rapidly fatal case of cholera. M. p. about 700.
(*a*) An epithelial cell.
(*b*) Lymph corpuscles, the interior of which contains large numbers of minute straight bacilli.
(*c*) A mass of the small bacilli and a few commas.
(*d*) Commabacilli and small straight bacilli embedded in the hyaline mucous interstitial substance.

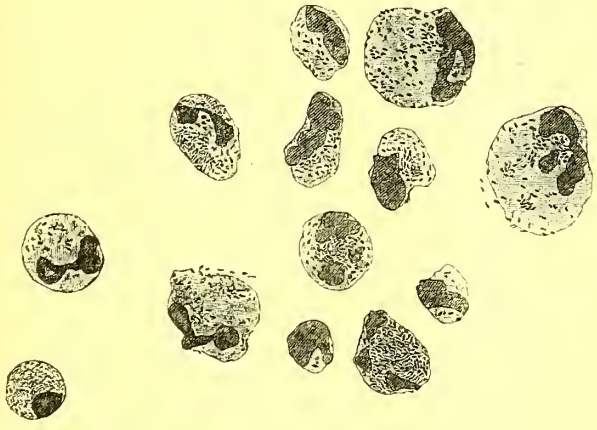


Fig. 5. From a preparation of fresh mucus flakes of the ileum of another typical rapidly fatal case of cholera. M. p. about 700.
Lymph corpuscles containing the minute straight bacilli.

Fig. 6. From an artificial cultivation of choleraic mucus flakes on damp linen. M. p. about 700.
Commabacilli of the ordinary type. Amongst them are some much thicker and including a vacuole.

Fig. 7. From a recent artificial cultivation of choleraic commabacilli in alkaline Agar Agar jelly. M. p. about 700.

Fig. 8. From an artificial cultivation of choleraic commabacilli on neutral Agar Agar jelly at ordinary temperature, after a few weeks. M. p. about 700.

Some of the commabacilli have become converted into circles, which by division give origin to two semicircular commabacilli.

Fig. 9. From a similar preparation as in previous figure.

Fig. 10. From a similar preparation.

Fig. 11. From an artificial cultivation of fresh mucus flakes of the ileum on damp linen. M. p. about 700.

Showing minute straight bacilli, commabacilli, and fine spirilla—spirillum tenue.

Fig. 12. From a preparation of fresh mucus flakes of the lower part of the ileum of a typical rapidly fatal case of cholera. (Duration of illness nine hours and a half, post mortem examination after one hour.)

M. p. about 700.

The forms here delineated are met with in the same mucus flakes.

(*a*) Masses of minute commabacilli.
(*b*) Masses of typical choleraic commabacilli.
(*c*) Minute circular and semicircular commabacilli.
(*d*) Large thick commabacilli.
(*e*) Masses of the minute straight bacilli.
(*f*) Micrococcus and thick straight bacilli.

Fig. 13. From a preparation of mucus flakes of the lower ileum having been allowed to undergo putrefaction for three days. M. p. 700.

Fig. 14. Artificial cultivation of choleraic commabacilli in alkaline gelatine peptone.. M. p. 700.

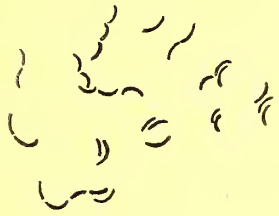


Fig. 15. Artificial cultivation of the same commabacilli as in preceding figure in alkaline gelatine peptone broth. The commabacilli are not so large and not so well curved. M. p. 700.

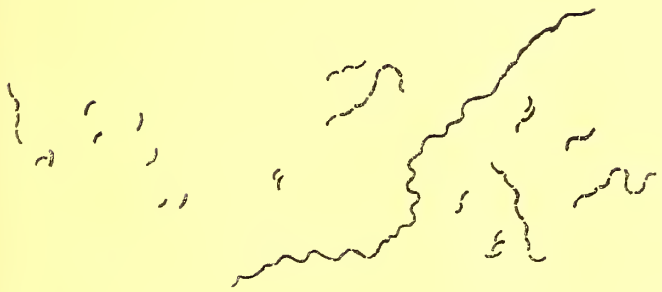


Fig. 16. Artificial cultivation of the same commabacilli in alkaline beef broth. M. p. 700.

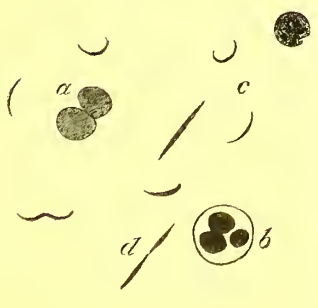


Fig. 17. From a preparation of blood of mouse. M. p. 700 See p. 22.
(*a*) Red blood discs.
(*b*) White blood corpuscle.
(*c*) Commabacilli.
(*d*) Long pointed bacilli.

Fig. 18. From the intestinal mucus of the same mouse.
Showing: commabacilli, pointed straight baccilli, and numerous spirilla tenua.

Fig. 19. Cultivation of choleraic commabacilli in gelatine mixture contained in flat glass dish.
The inoculation has been done by drawing lines over the surface of the solid gelatine mixture with the point of a needle that had been dipped previously into a cultivation of commabacilli.
The two figures represent the growth of the commabacilli in two of the lines after five days.
The opac central spot is due to a precipitation of masses of commabacilli in the gelatine liquefied by the growth.

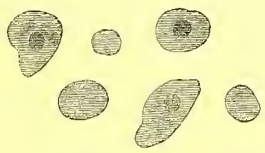


Fig. 19*a*. From a cultivation of choleraic commabacilli in gelatine in a glass dish, after inoculation in spots.

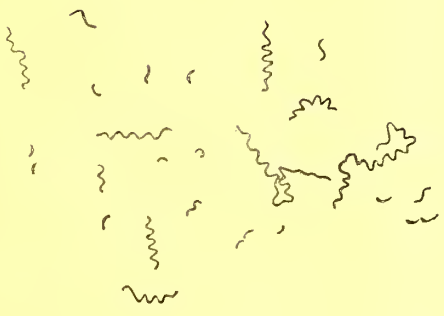


Fig. 20. From a cultivation of choleraic commabacilli in liquefied gelatine, after several weeks.

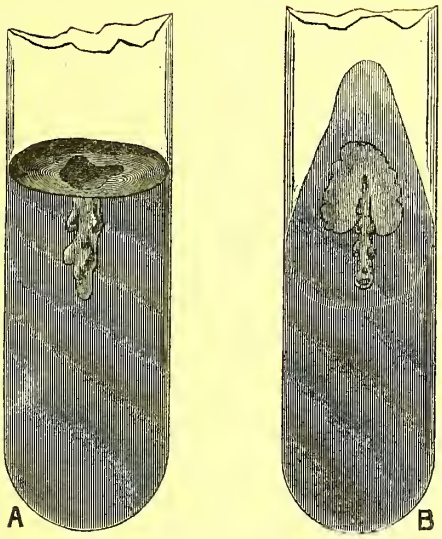


Fig. 21. From an artificial cultivation of the small straight bacilli of choleraic mucus flakes after several weeks.

A. In alkaline gelatine peptone broth.
B. In alkaline Agar Agar peptone.

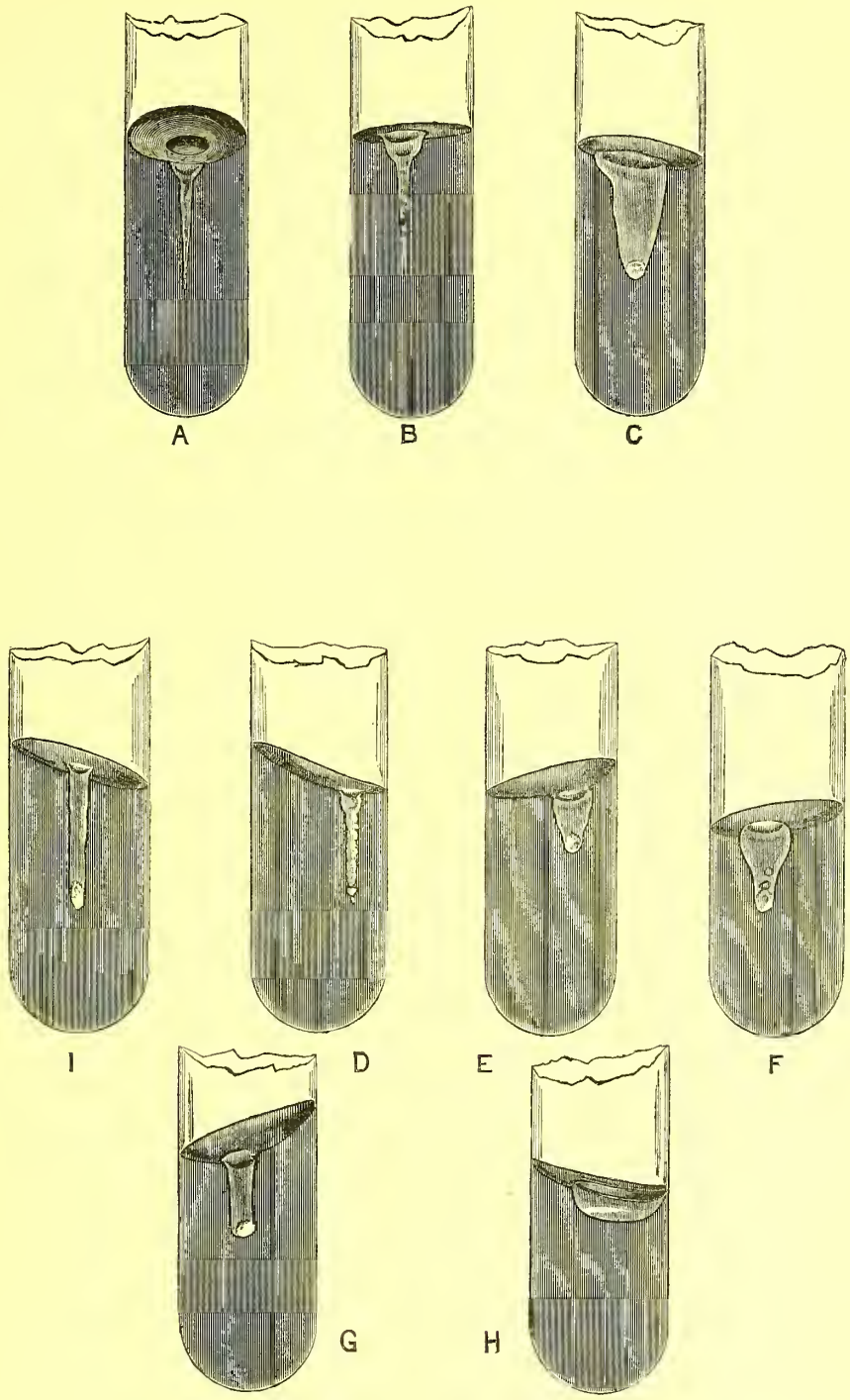


Fig. 22. (A) Cultivation, in solid gelatine peptone broth, of straight mobile bacilli of the fluid of the mouth after four days' growth, showing a funnel-shaped depression, the lower part of the funnel filled with liquefied gelatine containing the growth of the bacilli. Half profile view.

(B) Same tube viewed in profile.

(C and D) Cultivations in alkaline gelatine of choleraic commabacilli after five days' growth. In both tubes the inoculation had been made within a few seconds from the same stock. The surface shows the well known depression; the channel in which the inoculation was made contains the growth of the commabacilli, the gelatine is here liquefied. At the bottom of the channel is a whitish precipitate of masses of commabacilli.

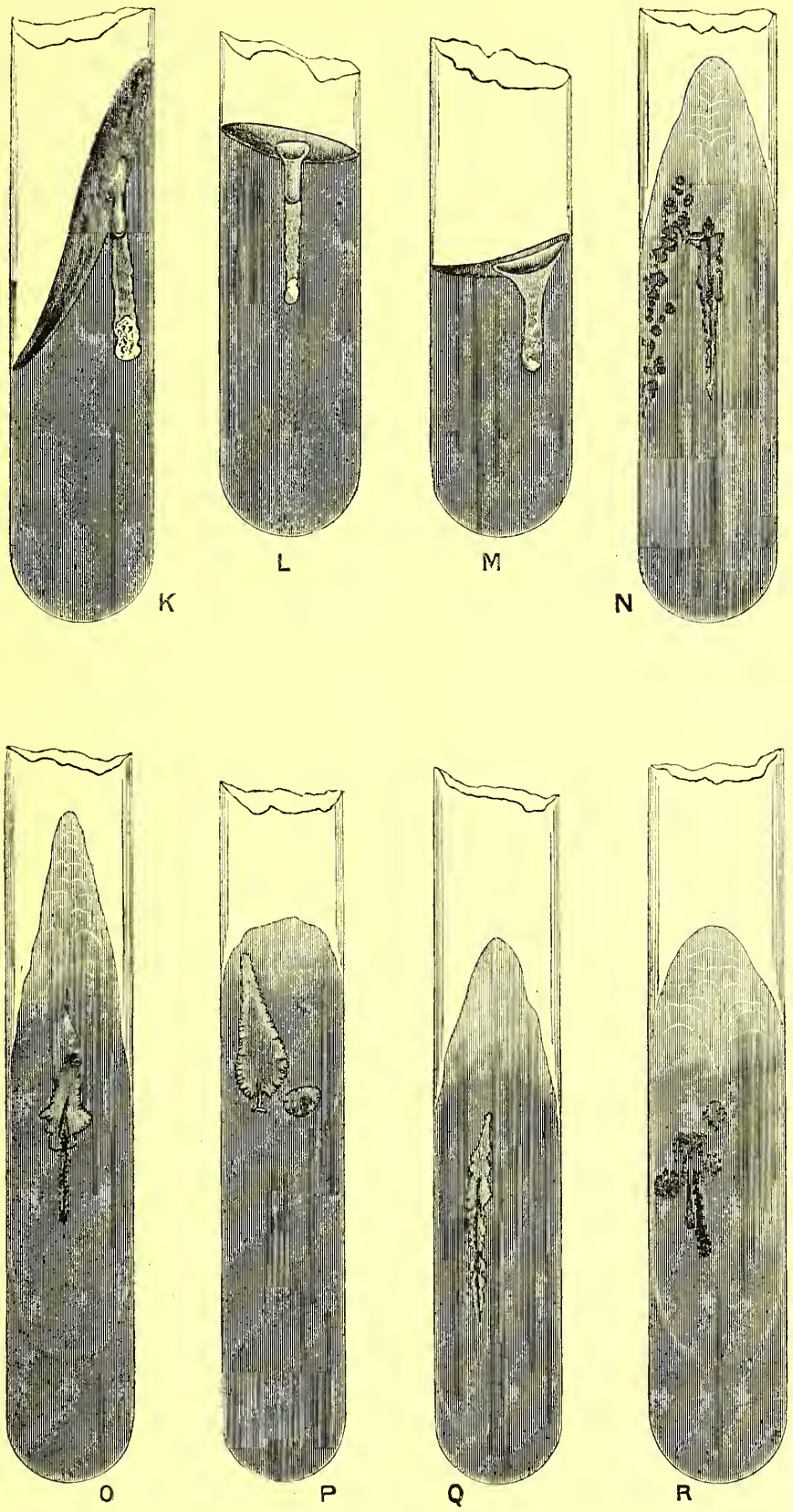
(E) Cultivation of commabacilli of the fluid of the mouth (healthy).

(F) Cultivation of choleraic commabacilli. In both E and F the medium is the same (alkaline gelatine peptone broth), and the inoculation was made within a few minutes.

(G) Cultivation of mouth commabacilli in gelatine peptone broth.

(H) Cultivation of choleraic commabacilli in alkaline gelatine peptone broth; the inoculation was made on the surface.

(I) Cultivation of choleraic commabacilli in alkaline gelatine peptone broth.



(K, L, and M) Cultivation of choleraic commabacilli in alkaline gelatine peptone.
In K and L an air bubble occludes the upper end of the channel of inoculation.

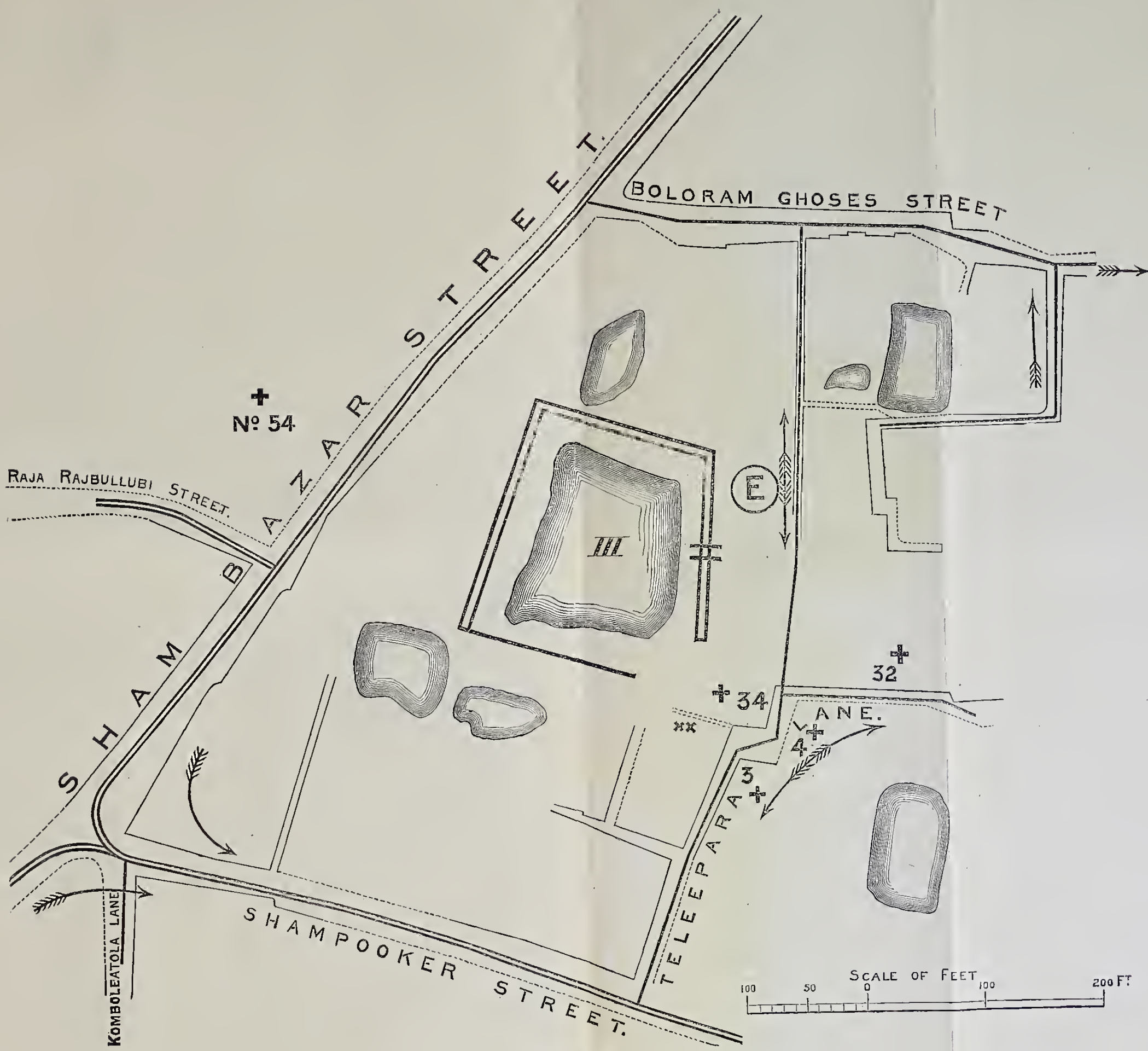
(N, O, and P) Cultivation of choleraic commabacilli in alkaline Agar Agar peptone and meat extract.

(Q and R) Cultivation of small straight bacilli of choleraic mucus flakes in alkaline Agar Agar peptone meat extract.

In Figs. 21 and 22, *i.e.*, cultivations in test tubes, only the part of the test tube is shown containing the (solid) nutritive medium.


II
I
N
NYA
BAZAR
16
GAS STREET
CANAL WEST ROAD.
99 FEET = 1 INCH
99 49½ 0 99 198 297 396 FT

PLAN I.


SHAM BAZAR STREET.
BOLORAM GHOSES STREET
No 54
RAJA RAJBULLUBI STREET.
III
E
32
34
LANE.
4
3
TELEEPARA
KOMBOLEATOLA LANE
SHAMPOOKER STREET.
SCALE OF FEET
100 50 0 100 200 FT

PLAN II.